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FURTHER STUDIES ON THE SYMPTOMS OF MANGANESE DEFICIENCY IN THE RAT AND MOUSE [1]

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In the interval since 1931 when the first papers (Orent and McCollum, '31; Kemmerer, Elvehjem and Hart, '31) appeared with clear evidence for the necessity of manganese, this element has been found essential for normal reproduction, lactation, bone formation, growth and the activity of certain enzymes (Shils and McCollum, '42). Although all reports agree that manganese deficiency symptoms occur in rats and mice, they disagree as to the nature of these symptoms. Workers at Wisconsin have reported decreased growth and disturbances of the oestrous cycle resulting in sterility (Kemmerer, Elvehjem and Hart, '31; Waddell, Steenbock and Hart, '31; Skinner, Van Donk and Steenbock, '32; Boyer, Shaw and Phillips, '42). Orent and McCollum ('31) found no differences in oestrus or growth but, rather, poor reproduction with very high mortality of young and poor lactation performance. Daniels and Everson ('35), on the contrary, found lactation and oestrus both unaffected but observed high mortality of the young as a result of congenital debility.

The experiments reported here were initiated to gain further information on the effects of the deficiency in the hope that the apparently contradictory results of the preceding

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work might be clarified. This has been partly accomplished in that certain findings have been confirmed and extended. In addition, a new symptom of manganese deficiency in the rat has been observed.

DIETS

We have devised a solid basal diet low in manganese (0.2–0.3 µg. per gram) yet allowing excellent growth when supplemented with this element. It is easily made and consists of whole milk powder [2] 20.00 parts; cottonseed oil U.S.P. 11.68; casein and dried yeast extract [3] 16.00; sucrose 45.00; salts [4] 2.82; also, per kilo, percomorph oil 20 drops, choline chloride 200 mg., and thiamine chloride 1 mg. The drinking water was distilled and contained 1 ml. of 8% potassium iodide solution per liter. We designate this diet as diet 1 in this paper. Diet 21 is a modification in that pure vitamins [5] replaced the yeast extract; casein was fed at a level of 16.00. Diet and water were given ad libitum.

With the cottonseed oil and milk fat giving a high caloric value per gram the average manganese intake of an adult rat on this diet was less than 3 µg. per day. This is a somewhat higher level of intake than that on a diet consisting of whole milk, Fe, and Cu, such as used by the Wisconsin workers. However, it is known that a whole milk regime is not entirely adequate (Orent and McCollum, '32; Van Donk, Steenbock and Hart, '33; Richardson and Hogan, '40).

[2] Spray-dried by Roger process — Ward's.

[3] Yeast extract. Anheuser-Busch yeast was stirred for 3 hours in 50% alcohol (500 gm. yeast in 5 liters of alcohol), allowed to settle and filtered. 500 ml. extract were air-dried on 150 gm. casein. The casein was acid-washed and alcohol-extracted or washed.

[4] Salt mixture: $K_2HPO_4 - 2250.00$; $CaCO_3 - 2340.00$; $Fe(NO_3)_3 \cdot 9 H_2O - 393.70$; $MgSO_4 \cdot XH_2O - 450.00$; $NaCl - 900.00$; Cu acetate $- 15.00$; $ZnSO_4 \cdot 7 H_2O - 15.00$. $Fe(NO_3)_3 \cdot 9 H_2O$ was purified by recrystallizing $Fe(NO_3)_3 \cdot 9 H_2O$ twice from 10:1 nitric acid (Cleaves and Thompson, J. Res., Nat. Bur. Stand., 18, 595, 1937), and once from water.

[5] The vitamins in diet 21 were, per kilo: thiamine 2 mg.; riboflavin 3 mg.; pyridoxine 3 mg.; calcium pantothenate 5 mg.; inositol 3 mg.; choline chloride 200 mg. The synthetic vitamins used in this experiment were generously supplied by Merck and Company.

The control animals received manganous chloride ($MnCl_2 \cdot 4 H_2O$) at a level of 0.18% in the diet for the first 6 months. The effect of manganese in producing rancidity became marked in hot weather and thereafter the manganese was omitted from the diet and added to the drinking water (100 mg. per liter). The regime with manganese is hereafter called diet 1 M.

The manganese content of the diet and various components was determined by a method essentially that of Skinner and Peterson ('30). The intensity of the permanganate color was determined in a Klett-Summerson photoelectric colorimeter using a glass absorption cell of 40 mm. Concentrations of manganese of 0.2 µg. per milliliter were detectable. By ashing relatively large quantities of diet (50–60 gm.), accurate determinations could be made. All solutions were brought to 50 ml. volume for colorimeter readings and the blank values were determined by adding 2–3 drops of 0.25% hydrogen peroxide solution directly to the solution in the colorimeter absorption cell to discharge the permanganate color (Rowland, '39).

RESULTS WITH THE RAT

Reproduction and lactation

Experiment 1 — Females. Forty stock females 3 weeks of age were paired according to litter into two groups. One group was placed on diet 1 alone; the other group received the basal diet plus manganese (diet 1 M). After 2 months, mating with fertile stock males was begun and continued periodically for over a year. The average number of litters obtained per animal was 3.4 with a minimum of 2 and a maximum of 7. There was a total of sixty-nine litters in the original deficient group; of these fifteen were born to seven animals given manganese after a long period on the deficient diet. The control group had seventy litters.

The pregnant females, when about to deliver their young, were placed in screenless reproduction cages with pans covered with filter paper clippings. These cages were examined twice daily and the young weighed when first seen.

Table 1 summarizes the reproduction history of the females in experiment 1.

Despite the fact that the viability records of the offspring of the control females did not equal those of stock rats, the difference between the viability performances of the young of the deficient and control groups is striking. Only four or 1.5% of the young born of deficient females were weaned and it is noteworthy that these four all occurred in one litter. All the

TABLE 1

Viability of offspring of females in experiment 1.

DEFICIENT FEMALES

Diet	Number of					Number and per cent of	
	Females	Litters	Young found alive	Young found dead	Young kept with mother (A)	Young (A) dying within 48 hrs. of birth	(A) weaned
1	20	54	372	51	250	232 (93%)	4 [1] (1.5%)
1 M	7 [2]	15	54	21	44	19 (43%)	21 (48%)
CONTROL FEMALES							
1 M	20	70	424	66	331	122 [3] (37%)	185 (56%)

[1] All four were in one litter.

[2] These seven females were originally on diet no. 1 and after several pregnancies were placed on diet 1 M.

[3] Of the 122 that died 57 were offspring of two females.

other 246 young in fifty-three litters died, 93% of them in less than 48 hours. In only a very few young was there any visible evidence of milk.

When manganese was given to seven of the deficient females (which had lost all their young in nineteen litters while on the manganese-low diet), half of their young were weaned. The percentage viability of this group closely approximates that of the control females. It is evident that manganese is necessary for the production of viable young and that this aspect of manganese deficiency is reversible.

Despite the fact that their young did not survive, the behavior of the deficient females appeared normal. They built nests in most pregnancies and hovered over and carried their young normally. They suckled and raised foster stock and control young. These observations on behavior of the deficient mothers are at variance with the observations of Orent and McCollum ('31). It was noted in some instances that the mother lost interest in her young, but this apparently occurred only when they did not suckle.

The failure of many of the young of the control animals to live cannot be explained at present. It is of interest to note, however, that the results of the individual females varied widely. Seven females weaned the young of all their litters and only two lost all their young; two of the twenty females were responsible for 47% of the non-viable young. That the results were not due to a borderline deficiency of the B complex is indicated by the fact that increasing the yeast extract or synthetic B vitamin content of the diet had no effect on the viability of the young of either group.

Experiment 1 — Males. Twenty-four males were placed on diet 1 at weaning and sixteen littermates of the same sex were placed on diet 2 M at weaning. No significant differences in growth, sperm motility, or testicular weight were noted over a period of 14 months.

Exchange of young

The results obtained upon exchanging and transferring young were similar to those of Daniels and Everson ('35). Eighty-three deficient young from seventeen litters were transferred to stock or control lactating females within 24 hours after birth but only three of these lived to 21 days. When eighty-four stock and control young from twenty-three litters were given to manganese-deficient mothers, forty-nine lived 21 days. Thus the deficient mothers were able to raise foster young while their own young died. In most instances where there was failure of a deficient female to suckle foster

young, the failure occurred with first litters. With subsequent litters the foster young were weaned in almost all cases.

Fertility and oestrous cycle

Observations were made on fertility and oestrous cycle of the females of both groups throughout experiment 1. For the first 10 months on the diets both groups exhibited a normal oestrous cycle and reproduction. Thereafter a prolongation of the oestrous cycles was frequently but not always found, with the affected females remaining in the postoestrum or anoestrum stages of the cycle for weeks. Mating did not occur. There appeared to be no significant difference between the deficient and control groups.

Reversibility of manganese deficiency in females

The symptoms of manganese deficiency in females can be overcome by the administration of this element even after 428 days on the deficient diet. As indicated in table 1, upon manganese administration the percentage of young weaned changed from 1.5 to 48, the latter figure approximating that of the females of the control group.

Development of manganese deficiency in adult females (experiment 1 A)

Manganese deficiency can be produced in adult females. In this experiment eleven stock females 5 to 6 months of age were used after five had been tested for fertility on stock diet.

When these females were mated, maintained on stock diet until near the end of pregnancy, and then placed on the deficient diet, the young that were born appeared normal. However, when these young were weaned and divided into two groups, one group on the deficient diet and the other receiving manganese, marked growth differences became apparent, particularly with the males. Continued mating of the adult females on the deficient diet resulted in increasingly poor viability performance of their offspring. Many of these young

showed symptoms of ataxia, poor equilibrium, and weakness in the third week of life, and were markedly underweight. In still later litters all of the young died soon after birth.

Development of young

Almost all of the young of the deficient females in experiment 1 died soon after birth. No definite pathological changes were observed [6] in these young. The deficiency presumably results in an acute biochemical lesion in some system or systems of fundamental importance after birth. There appeared to be no significant differences between the birth weights of the deficient and control young.

The few young of the deficient females of experiment 1 which lived as long as 21 days were subnormal in weight and in addition exhibited an ataxia, incoordination and loss of equilibrium. The offspring of deficient females in other experiments which survived long enough almost invariably developed the same symptoms. These were apparent by the end of the second week of life and were characterized by rotational movements. Occasionally the affected animal walked in a straightforward manner but soon fell over on its side or back, righting itself with difficulty. The loss of equilibrium was marked and included loss of the righting reaction. Usually the animals were quite active. The symptoms increased in severity to about the eighteenth day, whereupon the animal either died or else recovered to a great extent; however a noticeable failure of the righting reaction not infrequently persisted.

This condition was best produced by developing the deficiency in mature females. The young of the second litter usually showed the symptoms. It is interesting to note that Caskey and Norris ('40) observed an ataxia in the offspring of manganese-deficient hens.

Skeletal development

Abnormal bone development in manganese-deficient rats has not hitherto been demonstrated with certainty, although

[6] Follis, R., Jr. and Shils, M. E., to be published.

the possibility of its occurrence is indicated by the report of Barnes, Sperling and Maynard ('41). It is well-known that in manganese-deficient chicks skeletal abnormality is the outstanding symptom.

Our preliminary results indicate that the surviving young of markedly deficient females develop skeletal abnormalities. The gross effect is a shortening and bowing of the forelegs. The pathological changes will be described elsewhere in detail.[6]

Growth

We have found that manganese is essential for optimum growth, thus confirming the observation of Boyer, Shaw and Phillips ('42). This was demonstrated in the offspring of depleted mothers (experiments 1, 1 A and 4) and also in first-generation rats on modifications of diet 1 (experiments 7 and 8).

In experiment 1 no decreased growth was noted in the deficient rats which were on diet 1 since weaning. However, in the few surviving offspring of this group, marked decreases in growth were noted (fig. 1). In experiment 1 A as well as in experiment 4 decreased growth was marked in the offspring of adult females placed on the deficient diet late in pregnancy.

Experiment 4. In this experiment stock females were placed upon the manganese-low diet (diet 1) during the last week of pregnancy and maintained on it during lactation. At 21 days the young which were all of good weight and appearance were divided into two groups as littermate pairs; one group received the manganese-low diet, the other the same diet plus manganese. Growth records on twenty males and sixteen females are shown in figure 2. The differences in growth between the deficient and the control animals are obvious and are especially marked with the males. The decreased growth of the deficient males is associated with sterility and poor appearance of the fur.

Just as the reproduction symptoms of deficiency in the female can be reversed by administering manganese, so the

decreased growth of deficient males and females can be reversed by giving manganese even after 38 weeks on the deficient diet (fig. 3).
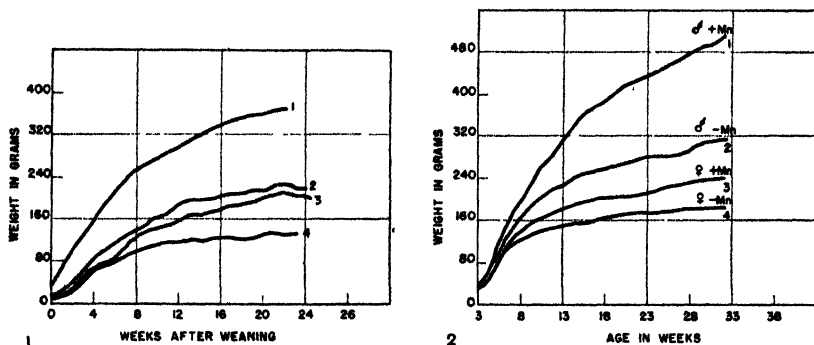
Fig. 1 The effect of manganese deficiency on growth of offspring (expt. 1). Curve 1 illustrates the growth performance of a typical male offspring of a control female on diet 1 M. Curves 2 and 3 are for the males and curve 4 for the female which were three of the only four deficient young to be raised to weaning by a deficient mother (table 1). The deficient rats also showed marked incoordination.

Fig. 2 The effect of manganese deficiency on growth (expt. 4). All the rats used in these experiments were the offspring of females started on the deficient diet during the last 4 to 7 days of pregnancy. At weaning the littermates whose weights are illustrated were paired, one-half being maintained on the deficient diet of the mother (diet 1) and the other half on the same diet supplemented with manganese (diet 1 M).

Curves 1 and 3 are, respectively, based on the average weights of ten males and eight females receiving manganese; curves 2 and 4 are those of like numbers of deficient rats. The differences are particularly marked with the males.
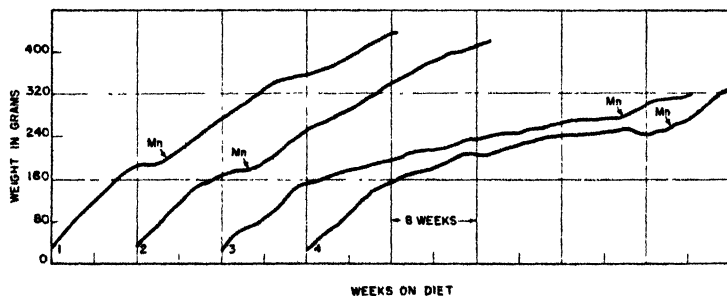
Fig. 3 The need and utilization of manganese for growth at various ages (expt. 4 C). Growth curves are given for four male littermate rats which were the offspring of a mother placed on the manganese-deficient diet a week before delivery. These young were continued on the deficient diet until manganese was added at the time indicated by an arrow.

These growth results indicate that manganese is needed by the rat not only during reproduction and lactation, but also during the period of rapid growth, and that it can probably be utilized and possibly is necessary at all stages of life.

Sterility in deficient males

The occurrence of sterility and testicular atrophy in manganese-deficient males was first noted by Orent and McCollum ('31) and has been confirmed by others. We have likewise been able to produce sterility and testicular atrophy in the great majority of the males of experiment 4, that is, in males whose mothers were on the deficient diets during pregnancy. No testicular atrophy was noted in the males in experiment 1, although there were histological changes.[7]

Effects on growth of changing the Ca and P levels in the manganese-low diets (experiments 7 and 8). It is well known that increasing the Ca or P levels in the diet of chicks on low manganese diets accentuates the appearance of perosis (Wilgus and Patton, '39). In addition, the Ca:P ratio of the manganese-low diet used by Orent and McCollum ('31) was calculated at 0.46; whereas the Ca:P ratio in our diet was 1.4.

In order to determine (a) whether more severe symptoms could be induced by altering the Ca:P ratio and (b) whether the differences in Ca:P ratio between the two manganese-low diets of Orent and McCollum ('31) and that described here

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[7] The modifications from Baldwin ('35) were, briefly: The use of a glycine buffer pH 9.5 instead of NaOH with which to make the liver brei. A few milliliters of the buffer were used to grind liver to a paste and then the rest of 25 ml. of the buffer used, together with distilled water to bring volume to 100 ml. The flasks were shaken 7–10 minutes and filtered through filter paper on a Buchner funnel. This procedure was found more desirable since the brei did not become gelatinous, and allowed more certain adjustment of pH: 2.5 ml. brei, 0.5 glycine buffer, and sufficient water or manganese solution added to bring volume to 5.1 ml. Suitable blanks were used. The substrate was 2.0 ml. of 5% arginine carbonate (Eastman) or arginine monohydrochloride (Eastman). The monohydrochloride solution was made up with sufficient NaOH to neutralize the HCl calculated to be present with the arginine. The reaction was stopped after 30 minutes at 28°C. and the tubes heated in boiling water. The contents were diluted to 25 ml. without filtering and suitable aliquots used for urea determinations by the method of Krebs and Henseleit ('32) using a suspension of Squibb's urease tablets.

could account for the differences in results the following experiments were performed.

Experiment 7. The P content of diet no. 1 was increased by the addition of K_2HPO_4 until the Ca:P ratio was 0.46 (that of Orent and McCollum's diet, '31). Four females and six males (stock weanlings) were placed on the manganese-deficient "high" P diet (diet 7) and five females and five males (littermates) were placed on the same diet but received manganese in the drinking water.

The growth and reproductive behavior of the females were the same as had been observed on the diets without added P. The deficient females consistently lost their young, whereas the controls weaned many of theirs. There were no significant differences in growth.

With the males, however, definite differences in growth were observed (fig. 4) in all instances. After 36 weeks on the deficient diet, the animals responded to manganese (fig. 4).

Experiment 8. The content of the original diet 21 was increased by the addition of $Ca_3(PO_4)_2$ so that the Ca was raised from 0.614% to 1.5% and the P from 0.46% to 0.9%, with a change in the Ca:P ratio from 1.4 to 1.66. Two littermate groups of six rats each were used, one receiving the deficient diet (diet 28) and the other receiving diet 28 plus manganese. Here again very significant differences were noted in growth between the two groups. The deficient males responded to manganese after 5 months (fig. 5).

Inasmuch as no growth differences were noted in males on the original diet, the differences in experiments 7 and 8 seem ascribable to the increased amounts of P alone and Ca and P together in the diets. As found in the work with chicks (Wilgus and Patton, '39), the increased amounts of Ca and P intensify the symptoms of manganese deficiency, and in all probability act in a similar fashion by rendering less available the manganese in the diet.

RESULTS WITH MICE

Mice were placed on the experimental diets (1 and 1 M) soon after weaning. Reproductive results indicate that the

mice behaved like the rats. Four females on the deficient diet had a total of ten litters with sixty-one young, all but six of which died within 48 hours. None lived more than 3 days. Five control females on the same diet receiving manganese in the drinking water had fourteen litters with ninety-five young, thirty-three of which were weaned.
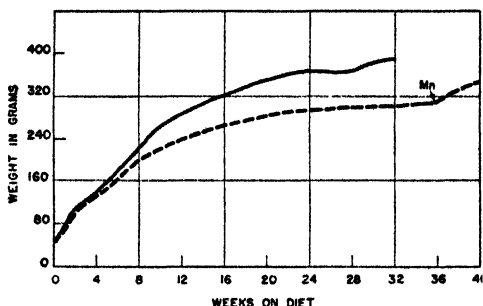
Fig. 4   The need and utilization of manganese for growth (expt. 7). Stock males were placed at weaning on manganese-deficient diet 7 and as the broken-line curve (average for six rats) indicates, did not grow as well as littermate controls receiving the same diet plus manganese (solid line, average for five rats). The deficient rats were able to respond to manganese after 36 weeks. Diet 7 had an increased phosphorus content over that of diet 1, on which no decreased growth was noted.
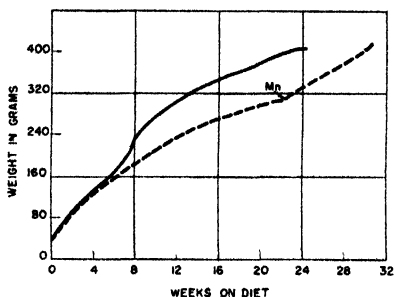
Fig. 5   The need and utilization of manganese for growth (expt. 8). Stock males were placed on deficient diet 28 at weaning (broken line) and showed decreased growth in comparison with littermate controls receiving manganese. The deficient animals gave a marked response to manganese after 22 weeks. Curves are averages for six rats. Diet 28 had a higher Ca and P content than diet 1.

ARGINASE ACTIVITY

The reports of Edlbacher and Pinosch ('37) and of Richards and Hellerman ('40) on the activation of manganese of liver arginase preparations led naturally to the measurement of arginase activity in manganese-deficient rats. Arginase has assumed prominence since Krebs and Henseleit ('32) demonstrated its role in the formation of urea.

The procedure used for the determination of the activity of the enzyme was a modification of the method of Baldwin ('35).[7] One gram samples were used in all instances to make the liver brei and only one concentration of this brei was used in the determination of arginase activity. All results are expressed as milligrams of urea formed in 30 minutes by 1 mg. dry weight of liver. The moisture and fat contents of the livers of the deficient and control rats were the same.

That the method gave reproducible results is indicated by numerous determinations of enzyme activity on three samples, each done in duplicate, from the same liver. In no instance was the variation more than $3\frac{1}{2}\%$ from the average.

Results

Arginase activity has been determined on fourteen deficient and thirteen control rats and on several stock animals; their ages varied from 10 to 16 months. The results have been quite consistent for each group and representative figures are given in table 2.

It is evident (a) that there is a decrease of approximately 50% in the arginase activity in the livers of the deficient rats; (b) that the addition of manganese increases the activity in both groups and raises the activity in the deficient group to that of the controls, this latter point being evidence that the formation of the protein component of the enzyme is not affected by deficiency of manganese; and (c) arginase activity in the females is appreciably lower than that in the males in both groups.[8]

[8] Others (see Lightbody, '38) have previously noted this sex difference. However, Lightbody ('38) found in his series of rats that by the 200th day of life the sex difference had disappeared; in our study the difference was still marked in rats 480 days old.

In several instances where arginine monohydrochloride rather than arginine carbonate was used as substrate, a similar decrease in activity of the liver preparation from the deficient rats occurred. However, it is noteworthy that with arginine monohydrochloride as substrate the addition of manganese, although it increased the activity in both groups, did not restore that of the deficient group to the level characteristic of the control rats. These results, considered without those with arginine carbonate as the substrate, might have led to the conclusion that the enzyme concentration as well as

TABLE 2

Arginase activity in the liver of manganese-deficient and control rats.
(5% arginine carbonate as substrate.)

CONTROL RAT NO.	ARGINASE ACTIVITY [1]		DEFICIENT RAT NO.	ARGINASE ACTIVITY [1]	
	No added manganese	60 μg. manganese added		No added manganese	60 μg manganese added
21 B ♂	0.930	1.826	21 A ♂	0.642	1.832
22 B ♂	1.230	1.687	22 A ♂	0.661	1.770
29 B ♂	1.016	1.593	29 A ♂	0.548	1.524
2 D-22 B ♂	1.182	1.509	2 D-22 A ♂	0.548	1.433
15 B ♀	0.896	1.684	1 A ♀	0.406	1.331
Average	1.051	1.660	Average	0.561	1.558

[1] Expressed as milligrams urea formed in one-half hour by 1 mg. dry weight liver at 28°C.

the activity was decreased in the deficient livers. Boyer, Shaw and Phillips ('42) used arginine monohydrochloride and likewise found that manganese did not restore the liver arginase activity of manganese-deficient rats to that of the controls. They concluded that the decreased activity "represented differences of enzyme concentration and not of activation." Our results with the carbonate do not support this conclusion; however, we cannot account for the differences between the two substrates, except perhaps as an effect of CO_3^- or Ce^-.

The finding of a decreased liver arginase activity in vitro raised the question as to whether decreased arginase activity

in vivo could account for the symptoms observed in the deficient animals. Studies were initiated on the growth and symptomatology of young manganese-deficient rats given ammonium citrate at a level of 5% in the diet. Since arginase is concerned with the formation of urea (Krebs and Henseleit, '32), it might be expected that an increased burden on the urea-forming system might result in an accentuation of symptoms. In a preliminary experiment, eighteen young born of partially deficient females were divided into three groups. One group received the manganese-low diet, the second group the manganese-low diet plus 5% ammonium citrate, and the third group the manganese-low diet plus manganese plus 5% ammonium citrate. In 9 months no significant differences were observed between the deficient animals receiving the ammonium citrate and those not receiving it, although both of these groups did not grow as well as the group receiving manganese. This supports the observation (Boyer, Shaw and Phillips, '42) that there is no difference in nitrogen excretion in manganese deficiency.

A decreased activity of arginase found in vitro may not occur in vivo or, if it does, it may not affect the animal adversely until a still greater decrease occurs. More work is needed in an attempt to link decreased arginase activity to the symptoms of manganese deficiency. The finding of Lightbody ('38) is of interest in this connection in that he found a very great increase in arginase activity in the livers of rats soon after birth, that is, at the time when most of our manganese-deficient young died.

DISCUSSION

The findings reported here, together with those of other workers already mentioned, indicate that the symptoms of manganese deficiency constitute a complex both interesting and far from understood. The fact that the congenitally debilitated young show no histologic abnormalities is an indication of an acute "biochemical" lesion. The evidences of incoordination and poor equilibrium implicate manganese in

the functioning of the nervous system. The bone changes indicate that in rats as well as chickens manganese plays a role in skeletal formation and Ca and P metabolism.

The conflicting reports in the literature are challenging and we shall attempt to reconcile at least some of them.

Female reproductive effects. The differences between the Hopkins and Wisconsin workers may indeed be caused by the differences in diets. Milk as the main component is lower in manganese than the more complex solid diet used by us. Consequently the effect on the oestrous cycle may be the result of a more acute deficiency.

Since the literature on manganese deficiency appears so contradictory we think it worth while to summarize the points of agreement.

(a) Our finding and that of Boyer et al. ('42) that on a given diet rats and mice behave alike indicate no species differences and are further support for the belief that differences in observations in various laboratories are due to the variations in manganese levels of the diets.

(b) There is agreement that manganese deficiency (probably of a particular quantitative degree) results in congenital debility of young (our results; part of Orent and McCollum ('31) and Daniels and Everson, '35).

(c) It is agreed that manganese is essential for growth of rats and mice (our findings; Kemmerer, Elvehjem and Hart, '31; Boyer et al., '42).

(d) All work with rats is in agreement that the deficiency results in testicular atrophy and sterility.

(e) There is a decrease in arginase activity in vitro. We agree with Boyer et al. ('42) that there is decreased activity but not that there is decreased formation and concentration of the protein part of the enzyme.

(f) Our findings on bone changes are in agreement with the findings of manganese-deficient chicks.

(g) Our finding of a need for manganese for normal nervous system functioning is in agreement with observations made on manganese-deficient chicks.

There are some differences not so readily explained. The inability of the deficient females in the Orent and McCollum experiment ('31), in eight out of ten cases, to suckle stock or control young is at variance with our findings and those of Daniels and Everson ('35). Likewise we were unable to note any marked difference in the behavior of the deficient females toward their young.

From all this we conclude that it is apparently possible to develop three distinct stages of manganese deficiency in the female. In the least severe stage, the deficient female gives birth to viable young which manifest symptoms of incoordination, paralysis and poor equilibrium that are most marked in the third week of·life. In the second and more severe stage, non-viable young are born which die very shortly after birth. In the third and most severe condition, the oestrous cycle is disturbed to the point where sterility results.

SUMMARY

1. A solid basal diet has been devised which is very low in manganese and which when supplemented with this element allows much better growth than any manganese-low diet heretofore devised.

2. Deficiency of manganese in first generation female rats started on the manganese-low diet at weaning resulted in the production of non-viable young. The deficient females were capable of raising foster stock or control young, although their own offspring died. No abnormalities were noted in the behavior of the deficient females towards their young. No disturbance in oestrous cycle due to manganese was noted in first generation females. Deficient mice likewise gave birth to non-viable young.

3. No decrease in growth was noted in the deficient rats of the first generation on the basal diet. However, the deficient offspring, particularly the males, of stock females placed on the deficient diet late in pregnancy, were inferior in growth to littermates given manganese. Likewise, increasing the calcium and phosphorus contents of the basal diet resulted in a marked

decrease in growth of first generation males. Manganese is a factor necessary for optimum growth. The deficient males were sterile.

4. A hitherto undescribed symptom of manganese-deficiency was observed in young rats, characterized by loss of both equilibrium and coordination.

5. The symptoms of manganese deficiency, namely, production of inferior young and poor growth of the female and sterility and poor growth of the male, are reversible by administration of manganese.

6. A marked decrease in arginase activity of the livers of the deficient rats has been noted.

7. The observations of various authors on the effects of manganese deficiency are discussed in connection with these results.

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THE ROLE OF DIETARY PROTEIN IN HEMOGLOBIN FORMATION [1]

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The fact that hemoglobin is protein in nature led to the early belief that an adequate amount of dietary protein is essential for its formation. Indeed, Voit (see Jackson, '25) believed that the hemoglobin content of the blood varies to some extent with the protein content of the diet, and Rubner ('19) suggested that much of the widespread anemia in Germany following World War I was due in part to an inadequate intake of protein. Pernicious anemia was once believed to be related to "faulty digestion" (Fenwick, 1877), a view which prompted Minot and Murphy ('26) to study the effect of the treatment of the disease with a protein and iron-rich (liver) diet. The classical work of Castle ('29) may be interpreted as a further suggestion of a possible derangement in the metabolism of some protein derivative in pernicious anemia, a view which cannot be disregarded today because of the alleged peptide nature of the anti-pernicious anemia factor.

Experimental studies (Jencks, '22; McCay, '28) on rats and dogs with hemorrhagic anemia have given some indication that there is a relation between dietary protein and hemoglobin formation. The rate of hemoglobin regeneration after hemorrhage was found to be more rapid in animals allowed an adequate protein intake. However, the possible importance of concomitant variations in the intake of calories and minerals was not determined in these investigations. Studies in this laboratory (Orten and Smith, '37; Orten and Orten, '39) have demonstrated that a mild chronic anemia develops in rats fed a low-protein diet in contrast to a normal blood picture in controls given an adequate dietary protein intake. The work of Whipple and his collaborators (Hahn and Whipple, '39) has shown that in dogs maintained in a constant state of anemia from hemorrhage, more hemoglobin is formed when the diet contains an adequate amount of protein than when the protein content is low, even though the iron intake is adequate. Further evidence that dietary protein is involved in hemoglobin production is found in the observation (Bethell, '36) that the anemia of pregnancy in human subjects frequently may be due to a lack of adequate dietary protein and may be benefited by increasing the intake of protein.

The present investigation was designed to study further the relation of the energy intake and of iron to the chronic anemia produced in rats by the feeding of a diet low in protein.

EXPERIMENTAL

Twenty-one day old weanling female rats of the Connecticut Agricultural Experiment Station strain, weighing from 40–50 gm., were used. They were housed in individual cages having a wide mesh screen bottom. Five groups of animals were studied. Group 1, "adequate protein," received a synthetic diet consisting of 18% lactalbumin,[2] 10% sucrose, 41% white dextrin, 27% hydrogenated vegetable oil, and 4% salt mixture (Wesson, '32). The food consumption was recorded

[2] "Labco" lactalbumin, The Borden Company, Bainbridge, N. Y.

every 4 days; the average daily food intake for each rat of this group over a period of 76 days was found to be 8.8 gm. Group 2 received a "low-protein" diet having the same composition as the "adequate" diet except that it contained 3.5% lactalbumin and 55.5% dextrin. The average daily food consumption of rats of this group was found to be 4.9 gm.

Group 3, a "calorie weight" control group, received the same allowance of calories daily as was consumed by the "low-protein" group of rats (group 2), but a daily protein intake equal to that of the "adequate protein" rats (group 1) of the same weight. For example, during one typical 4-day period the amount of food given the "calorie-weight" controls was 19.2 gm., the average amount consumed by the low protein (group 2) animals during the same age-period. The average body weight of the "calorie-weight" animals at this time was 76 gm. When the adequate protein animals (group 1) weighed 76 gm. they consumed an amount of diet in 4 days which contained 5.9 gm. of protein. Therefore, the "calorie-weight" animals were fed a diet during this particular period so prepared that 19.2 gm. contained 5.9 gm. of lactalbumin (30.9% of the diet). The diet, of course, contained less dextrin but exactly the same amounts of fat, sucrose, and salt mixture as 19.2 gm. of the low-protein diet. Obviously, it was necessary to reconstruct this diet every 4 days. During the entire experiment the percentage of protein varied from 20.6 to 35.2, with an average of 31.0%.

Also, an "inanition control" group of rats (group 4) was studied by the paired feeding technique. These animals were paired with litter mates of the low-protein group and received the same daily amount of calories (and minerals) as did their low-protein pairs; but they received more protein since they were fed iso-caloric amounts of the "adequate protein" diet, containing 18% lactalbumin.

In order to further study the possible effect of calories, a diet of increased caloric value but of low protein content was fed to a small group of animals, "high-calorie" (group 5). These rats were given the average amount of low-protein diet

consumed by the rats of group 2 during each 4-day feeding period and in addition fat and sucrose in such amounts that the total number of calories ingested would be equal to that consumed by the "adequate protein" animals of group 1 during the same period. The fat and sucrose were added in the same proportions in which they appeared in all of the other diets.

All animals received the following vitamin supplements: 200 mg. of a rice polish extract [3] and 100 mg. liver extract [4] no. 343 daily, and 2 drops of cod liver oil [5] concentrate twice weekly. In preliminary experiments these quantities of vitamins were shown to be adequate in half these amounts; nevertheless full amounts were fed to further insure against a possible inadequacy of vitamins due to any altered requirement brought about by the protein deficiency. If an animal did not voluntarily consume all of the supplement, the remainder was force-fed.

At the end of the period of 76 days just described, the anemic, low-protein rats, together with additional similar animals started at a later date, were divided into three subgroups. One (group 2 A) was continued on the same low-protein diet. A second sub-group (2 B) was given a diet supplying the same average daily intake of calories as the "low-protein" group of rats had been consuming, but the protein content was increased to provide a daily protein intake equal to the average daily protein consumption of the "adequate protein" control rats (group 1). This made a diet containing 40% protein. The third sub-group (2 C) was continued on the low-protein diet, but was given 1.0 mg. iron as $FeSO_4$ daily as a supplement. This was added in solution to the vitamin supplements. Twelve of the group of "adequate protein" rats . (group 1) were continued as normal controls on their previous diet.

Hemoglobin determinations were made on blood obtained from a tail vein of all animals on the seventy-sixth day of the experiment and at the intervals thereafter indicated in the tables. A modified acid-hematin method was used and all readings were made in a photoelectric colorimeter calibrated with bloods on which the hemoglobin content had been determined by the oxygen capacity method.[6] Erythrocyte and reticulocyte counts were determined on the seventy-sixth day of the experiment. Bureau of standards certified pipettes and counting chambers were used in making the red cell counts. The reticulocyte counts were made on permanent smears stained with brilliant cresyl blue and counterstained with Wright's stain (Orten and Smith, '34). Body weights were determined weekly.

RESULTS AND DISCUSSION

The averaged results of the blood studies on the five groups of rats after 76 days on the experiment are given in table 1.

TABLE 1

Hematological findings in control rats and in rats fed a diet low in protein [1]

GROUP	DIET	NO. OF RATS	HEMOGLOBIN	ERYTHROCYTES	RETICULOCYTES
			gm./100 cc.	*M/cmm.*	*%*
1	Adequate-protein	15	15.8±0.7	8.1±0.6	2.2±0.2
2	Low-protein	26	11.5±0.9	8.1±0.9	7.3±3.5
3	Calorie-weight	8	15.7±0.6	8.8±0.4	2.2±0.6
4	Inanition control	8	14.8±0.7	8.8±0.2	2.2±0.5
5	High-calorie	4	9.9±2.1	7.5±1.4	16.1

[1] Group average values, together with standard deviations, obtained after the animals had been fed the various diets for 76 days.

The "adequate-protein" rats (group 1) showed hemoglobin values, erythrocyte counts, and reticulocyte counts which are normal for rats of that age (Orten and Smith, '37). The "low-protein animals" (group 2), on the other hand, consistently showed a mild chronic anemia, characterized by a distinctly

[6] Appreciation is expressed to Dr. Victor Schelling of Henry Ford Hospital for supplying bloods of known oxygen capacity content.

sub-normal hemoglobin content of the blood, a normal erythrocyte count, but an elevated reticulocyte count. The distinct reticulocytosis confirms the existence of a mild anemia in the animals fed the low-protein diet and, furthermore, indicates that there is a compensatory attempt by the bone marrow to increase the erythrocyte output. The "calorie-weight" control rats (group 3), given an abundance of protein but a restricted intake of calories had an essentially normal blood picture. This observation is convincing evidence that the anemia of the low-protein animals is due to an insufficient consumption of protein rather than to an inadequate intake of calories. This statement is further borne out by data obtained from both the "inanition controls" (group 4) and the "high calorie" rats (group 5). The daily caloric and mineral intakes of the inanition controls were the same as those of the low-protein rats (group 2) but the protein intake was greater, since the diet contained 18% protein. As is evident from the data in table 1, the inanition controls (group 4) showed an almost normal blood picture. It is to be noted, however, that the average hemoglobin level, while in the normal range, is less than that of the calorie-weight rats (group 3). This is undoubtedly due to the fact that the inanition control rats received less protein daily than did the calorie-weight animals, since the daily amounts of all other dietary constituents ingested by the two groups of rats were identical.

The data obtained on the "high-calorie" rats (group 5), given an adequate intake of calories but a restricted protein intake, demonstrate the inability of an increased consumption of calories to improve the hematological picture in rats fed the low-protein diet. In fact the animals of this group were even more severely anemic than were the "low-protein" rats, the hemoglobin and erythrocyte values being lower and the reticulocyte counts considerably higher. The increased severity of the anemia in this group may be due to the increased amount of fat in the diet, since a high fat diet apparently increases the rate of erythrocyte destruction (Loewy, Freeman, Marchello, and Johnson, '43). It was possible to

study only a few animals in this group because of the difficulties entailed in force-feeding the animals a relatively large amount of the diet each day.

The animals given the adequate protein diet showed an excellent rate of growth, attaining an average weight of 235 gm. at 97 days of age, which is greater than that of normal stock female rats of the strain employed (Smith and Bing, '28). The rats fed the low level of lactalbumin, on the other hand, grew very little, the average body weight being only 82 gm. at the end of the experimental period. The average weights of the "calorie-weight" control group (147 gm.) and the "inanition control" group (135 gm.) were greater than that of the low protein rats, showing that some of the added protein was undoubtedly being used to form tissue protein as well as hemoglobin. However, since the increase in body weight toward a normal value for rats of this age was relatively small in comparison with the increase of the hemoglobin level to practically normal, this observation may be interpreted as supporting the view (Whipple, '42) that hemoglobin synthesis occurs preferentially over body tissue protein formation. In contrast, the average body weight of the "high calorie" control rats (68 gm.), was even less than that of the "low-protein" group, despite the increased amount of calories ingested.

The results obtained from the hemoglobin determinations on the adequate-protein rats and various sub-groups of low-protein animals continued on the experiment are given in table 2. It is evident that the adequate protein control animals continued to maintain a normal amount of hemoglobin in the blood (Orten and Smith, '34) whereas the anemia persisted in the rats (group 2 A) continued on the low-protein ration and it became even more pronounced by the end of the experiment. The anemia has been found to increase progressively in severity in animals maintained on this regime for nearly 1 year.

When the low-protein rats were given the increased amount of protein (group 2 B), a remission of the anemia promptly

occurred. This took place in spite of an unaltered allowance of calories, iron, and other dietary constituents. The hemoglobin level in the blood of these rats became normal within 4 weeks after protein realimentation. This was accompanied by an average increase in body weight of 25 gm. On the other hand, increasing the iron intake of animals maintained on the low-protein diet (group 2 C), resulted in no consistent improvement. There was a slight transient rise in the hemoglobin level but at the end of the experiment the hemoglobin

TABLE 2

Hemoglobin content of the blood of control rats and of rats fed a diet low in protein supplemented with protein or iron [1]

GROUP	DIET	NO. OF RATS	HEMOGLOBIN — GM. PER 100 CC.					
			Initial	2 wks.	4 wks.	8 wks.	12 wks.	16 wks.
1	Adequate-protein (cont'd.)	12	16.5±0.3	16.6±0.3	16.3±0.2	16.7±0.2	16.1±0.2	16.2±0.2
2 A	Low-protein (cont'd.)	10	12.2±1.2	12.1±1.4	12.1±1.5	11.7±1.0	11.3±1.0	11.6±1.0
2 B	Low-protein + protein	12	11.5±1.7	14.9±0.2	16.1±0.2	16.1±1.0	15.4±1.0	15.5±1.0
2 C	Low-protein + iron	8	12.0±1.3	12.5±1.7	12.3±1.9	12.6±1.8	12.8±1.9	11.5±1.9

[1] Group average hemoglobin values, together with the standard deviations.

level of this group of rats was no greater than that of those given no added iron. The inability of added iron to increase to normal values the hemoglobin content of the blood of any of the low-protein animals indicates that a lack of iron is not primarily involved in the anemia of these rats. Also, since an unpurified iron salt was used there is no indication that the "trace elements," such as copper or cobalt, are involved. There was no beneficial effect of the supplemental iron on growth.

The foregoing experiments taken as a whole offer strong evidence that an adequate quantity of dietary protein is essential for normal hemoglobin formation in the rat and that the caloric intake is of minor importance. The mild chronic anemia occurred in all animals fed the low-protein (lactalbumin) diet, whereas a normal blood picture was found in rats given the same amount of diet containing an adequate quantity of protein but a restricted amount of calories. Furthermore, the chronic "low-protein anemia" developed in spite of an increased intake of calories. Further evidence that the observed anemia is due primarily to the low-protein intake was obtained in the group of "low-protein" rats realimented with an adequate amount of protein but with no change in the intake of calories, minerals, or other dietary constituents. A prompt remission of the anemia resulted in these animals.

The results obtained in the present experiment obviously give no insight into the mechanism involved in the development of the anemia in rats fed the low-protein diet. One logical explanation would be that a low protein intake simply decreases the formation of the body protein, hemoglobin. However, it is equally possible that the low protein intake may alter the absorption, retention, or utilization of some other substance, or substances, which is essential for normal hematopoiesis. Possible support to such an interpretation is the fact that rats fed a low-protein diet show diminished amounts of riboflavin in the liver, even though additional riboflavin is given (Sarett and Perlzweig, '43), and this vitamin appears to have a distinct effect on hematopoiesis in the hemorrhagic anemia of dogs (György, Robscheit-Robbins, and Whipple, '38). This question merits further careful consideration.

<div align="center">SUMMARY</div>

The administration of a diet low in protein (lactalbumin) but adequate in all other known respects produces a mild chronic anemia in rats.

The "low-protein anemia" may be prevented or cured by the allowance of an adequate protein intake without an

alteration in the amounts of calories, minerals, or vitamins consumed.

Increasing the intake of either calories or iron has no consistent beneficial effect on hemoglobin formation in the low-protein animals.

These observations warrant the conclusion that an adequate intake of dietary protein is essential for normal hemoglobin formation in the rat.

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THE EFFECT OF CONCENTRATION ON THE ABSORPTION OF VITAMIN A [1]

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There is considerable evidence that vitamin A is readily absorbed from the gastrointestinal tract. According to Clausen ('33) vitamin A in the blood reaches a maximum 3 hours after its oral administration, which would indicate either that a maximum rate of absorption has been attained by that time, or that the speed of storage and utilization has reached an equilibrium with the rate of absorption.

Baumann, Riising and Steenbock ('34) demonstrated that a marked disappearance of vitamin A from the intestine occurs within 3 hours after its administration while the vitamin A content of the liver reaches a maximum within 6 hours in spite of the fact that large amounts of the vitamin still remain in the gastrointestinal tract for as long as 12 hours after its ingestion.

Although the absorption of vitamin A may be decreased when taken in paraffin oil (Rowntree, '31; Andersen, '39), there is only a slight loss when mineral oil and butterfat are taken separately (Jackson, '31). The presence of bile in the intestine is not as important for the absorption of vitamin A as it is for that of carotene. Schmidt and Schmidt ('30) found that vitamin A could still be absorbed by choledochocolonostomized vitamin A-deficient rats in amounts sufficient to clear

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[1] Most of these data are from a thesis to be presented by A. G. Reifman to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Master of Science.

up the avitaminosis. Carotene, on the other hand, was ineffective in the treatment of such operated vitamin A-deficient rats (Greaves and Schmidt, '35), although when glycodesoxycholic or desoxycholic acid was added, the deficiency was overcome.

The vitamin A esters must first undergo saponification prior to absorption. According to the results of Gray, Morgareidge and Cawley ('40), an accumulation of vitamin A alcohol occurs in the gut wall at the height of absorption. In view of the fact that fat is absorbed more rapidly from the gut than vitamin A, these investigators believed that this vitamin does not play a major role in fat absorption. However, Lovern and Morton ('39) have shown that the rates of absorption of vitamin A and of neutral fat are parallel in the halibut but these results may not be applicable to higher animals since the halibut possesses no lymphatic system. The absorption of carotene but not of vitamin A is decreased in hens on a fat-free ration, according to Russell, Taylor, Walker and Polskin ('42).

The present paper is the first of a series of studies to investigate factors altering the absorption of vitamin A in the rat. The effect of concentration of the vitamin fed has been studied when concentrations varying from 100 to 1,000,000 I.U. per gram have been fed.

EXPERIMENTAL

The vitamin A solutions were fed by stomach tube to rats previously fasted for 48 hours and the amount of vitamin A remaining in the gut after 3 hours was determined. Rats from our stock colony were placed on a vitamin A-low diet [2] for 6 days prior to the fasting period in order to decrease the control level of vitamin A extractable from the gastrointestinal tract. The average control level was used as a correction factor. The procedures were similar to those employed earlier (Deuel, Hallman and Leonard, '40).

[2] Similar to the U. S. P. XI vitamin-A depletion diet except that unextracted casein was used.

Vitamin A solutions were administered in amounts of 300 mg. per 100 sq. cm. of surface area. Surface area was computed by the formula of Lee ('29) based on the weight after fasting. Absorption has been related to surface area rather than to body weight because this gives more uniform results (Deuel, Hallman and Leonard, '40). The vitamin A preparations contained approximately 100, 1,000, 10,000, 100,000 and 1,000,000 I.U. per gram. The three lowest concentrations were prepared by dilution of shark liver oil [3] of 110,000 I.U. potency with commercial hydrogenated cottonseed fat which already contained some vitamin A. For the highest levels the 110,000 I.U. shark oil and 1,004,000 I.U.[4] concentrate were fed without dilution.

After removal of the gastrointestinal tract, it was flushed with 70 cc. of freshly distilled diethyl ether. After drying with anhydrous Na_2SO_4 the extract was filtered into weighed flasks, and the Na_2SO_4 thoroughly washed with fresh ether. After removal of the diethyl ether by evaporation, the weight of the residue was determined. Except for the tests on the two highest concentrations, the residue was then saponified with alcoholic potash and extracted for 3 hours in a continuous all-glass extraction apparatus, using a low-boiling Skelly-Solve as the extracting agent. The extract was then made up to a suitable dilution with a high boiling petroleum ether fraction $(60°-80°C.)$,[5] and the vitamin A determined with the Beckman spectrophotometer at 323 mμ.[6] Because of the high dilutions required in the tests where the 100,000 and 1,000,000 I.U. material was used, the saponification was omitted. In the last tests (series II, III and IV), the extract was made up with redistilled isopropyl alcohol so that the final dilution was a 3-to-1 mixture of alcohol and petroleum ether; in these experi-

ments the spectrophotometer was read at 326 mµ which was the peak of the absorption.[7]

In order to determine whether the loss of vitamin A in the intestine might be ascribed to bacterial destruction rather than to absorption, tests were carried out in which the various preparations were introduced into the gut by stomach tube and the entire gastrointestinal tract removed as in our recovery experiments. After ligation of the esophagus, the gut was allowed to remain in an Erlenmeyer flask in an incubator at 37°C. for 3 hours, after which the contents were flushed out with ether in the usual manner. The foul odor which these preparations developed was an indication of the extensive activity of the putrefying bacteria in the intestine. Although much of the administered vitamin A remains in the stomach by this technic, the mechanical manipulation involved in the removal of the gastrointestinal tract causes considerable amounts of the administered fat to be forced into the intestine. However, in one series of tests (V), the fat was pressed out of the stomach at the start so that it would all be in the intestine during the incubation period. The various series of tests were carried on at different periods with different groups of rats. No absorption tests are recorded for series III because of the high control values for vitamin A in the intestine. Series V consists only of experiments designed to determine the extent of bacterial destruction of vitamin A. There is no difference in the significance of results of series I, II and IV but because they were carried out at different times and the sex of the animals was not the same, they are reported separately.

RESULTS

Table 1 records the various control tests, the recovery experiments, and the tests of recovery after subjecting the vitamin A preparations to the action of bacteria in the isolated gastrointestinal tracts.

[7] The authors wish to thank Fred Mattson for carrying out the determinations of vitamin A in series III, IV and V.

TABLE 1

The vitamin A and lipid recovered from the gastrointestinal contents of rats previously fasted for 2 days (A), or immediately after the administration of different concentrations of vitamin A in fat (B), or from the gastrointestinal tracts removed immediately after the administration of vitamin A but where the ligated G.I. tracts remained 3 hours in incubator at 37°C. before being flushed with ether (C).

SERIES	NO. OF TESTS	AV. BODY WT.	SEX	LIPID				VITAMIN A			
				Fed	Recovery			Fed	Recovery		
					Total	Corrected[1]	Per cent		Total	Corrected[1]	Per cent
Control tests (A)											
				mg.	mg.	mg.		I.U.	I.U.	I.U.	
I[2]	11	189	M	0	23.9	...	...	0	39.2	....	...
I	13	222	M	0	25.0	...	...	0	27.0	....	...
II	11	205	F	0	25.1	...	...	0	29.9	....	...
III	11	215	F	0	41.4	...	...	0	93.1	....	...
IV	18	136	M	0	18.7	...	...	0	36.7	....	...
Recovery when rats were killed immediately after fat administration (B)											
I	11	217	M	906	922	897	98.9	114	131	104	92.0
II	8	221	F	907	933	908	100.1	129	154	124	96.2
I	10	228	M	908	...	...	....	941	965	938	99.6
I	14	223	M	908	933	908	100.0	8,780	8,247	8,220	93.5
I	9	213	M	925	958	933	100.7	87,600	83,600	83,570	96.0
II	7	187	F	900	940	915	101.6	950,700	915,640	915,610	96.3
V	3	221	M	898	936	917	101.5	635	677	640	100.7
Recovery from gastrointestinal tracts after 3 hours' incubation (C)											
II	6	206	F	0	30.8	...	....	0	26.2	...	...
III	10	216	F	0	60.6	...	....	0	103	...	...
IV	7	172	M	0	34.7	...	....	0	55.8	...	...
II	16	198	F	886	946	921	103.9	123[3]	144	114	89.5
IV	5	175	M	907	915	896	98.8	185	200	164	88.7
I	6	202	M	906	927	902	99.5	114	129	102	89.5
I	4	203	M	908	918	892	98.3	941	987	960	101.9
I	2	202	M	908	932	907	99.9	8,780	8,737	8,710	99.2
I	3	226	M	927	959	934	100.7	87,600	82,730	82,730	94.4
II	2	156	F	850	873	848	99.8	904,500	869,000	888,970	96.2
V	3	253	M	898	925	890	99.2	635	679	642	101.0

[1] Corrected for amount found in gut contents of corresponding control group.

[2] Rats previously receiving stock diet were fasted 2 days. The stock diet is high in carotene and vitamin A. Other rats were kept for 6 days on carotene-free, vitamin A-low diet.

[3] Nine experiments only.

Although the average vitamin A present in the gastro-intestinal tract of animals fasted 2 days after being on the stock diet amounted to 39 I.U. which was an exceptionally large correction factor for the experiments with fat containing approximately 100 I.U. of vitamin A per gram, somewhat lower values of 27 and 25 I.U. were found following a preliminary period on a low vitamin A, carotene-free diet.

However, in a later series of tests on older female rats much higher control levels of both intestinal lipid and vitamin A were found (series III). The apparent vitamin A values and lipid contents were consistently parallel. Because of the high correction values of vitamin A (average 93 I.U.) which were extremely variable, all absorption tests from this group were discarded. Similar high levels (103 I.U.) were found in the incubated gastrointestinal contents of this group of rats. These data are included to indicate one of the difficulties in obtaining satisfactory control animals. The lowest control levels in intestinal lipid were found in series IV where the animals were subjected to a preliminary enema of 5 cc. of soap solution. Tests were started 3 hours thereafter. Comparable rats were used on the control, recovery, incubation and absorption tests in each series.

The recovery of lipid approximated 100% in all tests, while the average for vitamin A varied between 92 and 100%. Because of these high values, no correction is made for the recovery in these tests as in our former studies.

The action of the intestinal bacteria on the various vitamin A preparations did not lower appreciably the vitamin A recoverable from the gastrointestinal tracts after 3 hours. Moreover, there was apparently no concurrent synthesis of new vitamin A, as the results of the control tests showed no increase in experiments on female rats and only a slight absolute increase in the tests where male rats were employed.

Table 2 gives the average rates of absorption when the various concentrations of vitamin A were fed.

There is a progressive rise in the rate of vitamin A absorption from approximately 5 I.U. per 100 sq. cm. of body

surface per hour when 100 I.U. are given to 10,000 I.U. when 950,000 I.U. are administered. The absorption of the lipid is quite uniform at 35 mg. per 100 sq. cm. per hour except for the highest level of vitamin. In this case the material, which was a saponified concentrate of fish liver oil, probably contained little readily absorbable lipid.

TABLE 2

The absorption of lipid and vitamin A over 3-hour periods by rats fed 300 mg. of either hydrogenated cottonseed oil containing different concentrations of vitamin A, or vitamin A concentrates alone.

SERIES	NO. OF TESTS	AV. BODY WT.	SEX	FAT IN MG.				VITAMIN A IN I.U.			
						Absorbed				Absorbed	
				Fed	Recovered[1]	Total	Per 100 sq. cm. per hr.	Fed	Recovered[1]	Total	Per 100 sq. cm. per hr.
II	8	194	F	883	538	346	38.5	126	67	60	6.5
IV	7	151	M	758	453	305	39.8	155	125	31	4.2
I	11	198	M	887	580	307	34.5	110	64	46	5.2
I	9	203	M	911	589	322	35.8	941	683	258	28.5
I	9	200	M	888	559	329	36.6	8,600	5,320	3,280	369
I	9	250	M	958	665	293	28.5	90,590	68,880	21,710	2,108
II	5	183	F	900	873	27	3.1	950,100	865,400	84,700	10,140

[1] Corrected for control value.

DISCUSSION

The rate of absorption of vitamin A from the gastrointestinal tract of rats is directly proportional to the concentration of the vitamin administered. Vitamin A was found to be absorbed at a speed 2,000 times as fast when 950,000 I.U. were given as when 110 I.U. were fed. At the three lowest levels the amount of vitamin A absorbed varied between 38 and 27% of the quantity fed. This value dropped to 24% for the 100,000 I.U. material and to 9% for the 1,000,000 I.U. concentrate. No statistical evaluation is necessary to establish the different rates of absorption as no overlapping occurred in any case between individual experiments of the groups where different levels of vitamin A were given.

In these tests there is no evidence of a proportionality between the absorption of vitamin A and the fat as suggested by Lovern and Morton ('39). The quantity of fat absorbed over a 3-hour period varied from 39.8 to 28.5 mg. per 100 sq. cm. while the vitamin A absorption was varying from 4.2 to 2108 I.U. per 100 sq. cm. per hour. In the tests where the highest amount of vitamin A was absorbed, there was no evidence that lipid had disappeared. However, one would expect this to be the case since most of the lipid here is actually the vitamin itself and other constituents of the unsaponifiable residue.

There is no evidence that the vitamin A which disappears from the gastrointestinal tract is the result of destruction by bacteria in the intestine. When the various vitamin preparations were introduced into the stomach by stomach tube and the intestinal tract immediately removed and allowed to stand in flasks at 37°C. for 3 hours, the same quantitative recovery was obtained as when the material was flushed out of the gastrointestinal tract immediately, although considerable putrefaction had taken place. Moreover, the putrefaction of fish livers is without any marked effect on vitamin A content [8]; it has also been demonstrated that no carotene is destroyed by intestinal bacteria over a period as long as 24 hours.[9] These data are difficult to interpret in view of the report of Hickman et al. ('42) where it was shown that the administration of a tocopherol increased the growth response to vitamin A and carotene in vitamin A-deficient rats. Similar results were also obtained by Quackenbush et al. ('42). There is no direct proof that the apparent synergistic effect may be the result of a decrease in bacterial destruction. Also, Baumann, Riising and Steenbock ('34) concluded that large amounts of vitamin A were destroyed in the gastrointestinal tract of rats because no further increase in vitamin A in the liver occurred after 6 hours although large amounts of the vitamin were still in

[8] Personal communication of Alan Richardson.

[9] Unpublished work of Dr. E. Geiger reported at the meeting of the Southern California Section of the Society for Experimental Biology and Medicine at California Institute of Technology, January 21, 1943.

the gastrointestinal tract and none was subsequently excreted in the feces. Our experiments do not preclude the possibility that the destruction of vitamin A may proceed at an accelerated rate after 3 hours. However, another explanation of the experiments of Baumann, Riising and Steenbock would seem to be that the vitamin A continued to be absorbed after 6 hours and that such destruction takes place in the liver. The body must possess some means of destroying any excess of vitamin A since no satisfactory channel for excretion is available. That such an enzyme system may be augmented in activity so that carotenoid pigments also may be destroyed has been postulated by Mattson and Deuel ('43). The destruction in this latter case must necessarily take place after absorption.

The power of the rat to absorb vitamin A is remarkable. An average of 85,000 I.U. were absorbed in a 3-hour period when the most concentrated preparations were administered. Were the rat able to store this and use it efficiently, it would represent almost a 45-year supply at a daily consumption rate of 5 I.U.

SUMMARY

The rate of absorption of vitamin A in rats was found to be proportional to the concentration of the administered material. The average absorption per 100 sq. cm. per hour was as follows: 4.2 to 6.5 I.U. for 100 unit material, 28.5 for 1,000 unit, 369 for 10,000, 2108 for 100,000, and 10140 for 1,000,000 unit.

There is no relationship between the rate of absorption of neutral fat and vitamin A. Evidence is given which indicates that vitamin A is not destroyed by intestinal bacteria over a 3-hour period.

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NUTRITIVE DIFFERENCES IN RATIONS CONTAINING UNHYDROGENATED OR HYDROGENATED FATS AS SHOWN BY REARING SUCCESSIVE GENERATIONS OF RATS

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Kennedy and Palmer ('26) observed a nutritive difference between rations containing hydrogenated and unhydrogenated fat when they reported successful reproduction in three successive generations of rats on the Evans and Bishop sterility diet in which lard was replaced by a commercial hydrogenated fat. Confirmation and elucidation of these results were supplied later in reports by Evans and Burr ('27 b), Mattill ('27) and Olcott and Mattill ('34). Evans and Burr found that by increasing the lard quota from 8 to 22% in a ration consisting mainly of casein and starch the number of gestations resulting from first breedings was greatly decreased. These same authors showed that wheat germ, a known source of vitamin E, could be robbed of its effectiveness as a source of this vitamin when mixed with certain fats such as lard or oleic acid. Mattill ('27) associated this decrease in vitamin E activity with oxidative changes which accompanied the development of rancidity in unsaturated animal fats. He also reported unpublished work by Cummings in which animals were not sterile when the ordinary lard in sterility rations was replaced by hydrogenated lard. Cummings and Mattill ('31) confirmed the earlier observations of Evans and Burr ('27 a) which indicated an improvement in reproduction when hydrogenated cottonseed oil replaced cottonseed oil in the diet of

rats. A commercial hydrogenated vegetable shortening made by hydrogenating cottonseed oil to an iodine number of 70 was found to be an excellent and stable source of vitamin E (Weber, Irwin and Steenbock, '39). By comparing unhydrogenated cottonseed oil with hydrogenated cottonseed oil in rations otherwise the same, Cummings and Mattill ('31) found that hydrogenated cottonseed oil was the more reliable source of vitamin E. Olcott and Mattill ('34) reported that hydrogenation did not destroy the vitamin E in concentrates obtained from wheat germ oil and cottonseed oil. The protective factor against nutritional encephalomalacia of chicks was found to be present in hydrogenated cottonseed oil (Goettsch and Pappenheimer, '36). On a diet lacking vitamin E, resorption in the first pregnancy of mice was prevented by the administration of alpha tocopherol or by the addition of 5% hydrogenated cottonseed oil to the diet (Bryan and Mason, '40).

It has thus been demonstrated that vitamin E is present in hydrogenated cottonseed oil and in hydrogenated concentrates obtained from wheat germ oil and cottonseed oil. There are also indications that hydrogenation may result in a decreased tendency of vitamin E in the ration to be destroyed, presumably by oxidation. It appeared to the author that additional information pertaining to the relative nutritive effect caused by the presence of the unhydrogenated or hydrogenated types of fat in mixed rations could be obtained, not only by feeding the basal ration deficient in vitamin E, but also by employing rations of more adequate nutritive properties. Especially did it seem desirable to extend the study beyond the first gestation period, and, if animals were available, into succeeding generations. A study of this type would give information relating not only to fertility and reproduction, but also to the production of viable young and to lactation. For the purpose of studying the dietary properties of hydrogenated and unhydrogenated fats from the same source, it appeared that soybean oil could be chosen as an important example of an edible oil of relatively high susceptibility to oxidation.

EXPERIMENTAL PROCEDURE

In the above investigations the rations were composed of natural fats, processed fats, purified carbohydrate and protein, yeast as the carrier of vitamin B complex, and vitamins A and D in the form of cod liver oil and butterfat. These same constituents formed the basis for our present rations (table 1), but in addition a vitamin E supplement was simultaneously added in some cases as a check on the conclusion that vitamin E is destroyed in the ration containing unhydrogenated lard.

TABLE 1

Composition of rations.

	RATION A	RATION B [1]		RATION C [1]
Casein	30.0 [2]	18.0 [2]	Casein	8.0 [2]
Wheat embryo	2.0		Cottonseed meal	5.0
Dry yeast	4.0	8.0	Coconut meal	10.0
Dextrin	38.0	45.0	Yellow corn	47.0
Salt mixture	4.0 [4]	2.5 [5]	Whole milk powder	3.0
Cod liver oil	2.0	0.5	Cod liver oil	0.5
Agar		1.0	$CaCO_3$	1.0
Experimental fat	20.0	25.0	NaCl	0.5
			Experimental fat	25.0

[1] 3.0 cc. fresh milk daily; 3.0 gm. fresh liver weekly per animal.
[2] Alcohol extracted.
[3] Commercial.
[4] Steenbock no. 32 (Steenbock and Gross, '19).
[5] Hubbell, Mendel and Wakeman ('37).

In other experiments this basal ration was supplemented with fresh milk and liver in order to improve the dietary qualities of the ration.

With these rations a comparison was made between lard and hydrogenated cottonseed oil. Hydrogenated and unhydrogenated soybean oil were compared in a series of experiments where the basal ration was supplemented with fresh milk and liver and where the rations were composed largely of natural foodstuffs.

The hydrogenated soybean oil, which was a plastic product at room temperature with an iodine value of approximately 70,

came from the same lot of oil as the unhydrogenated soybean oil. This oil had previously been alkali refined and steam deodorized. The hydrogenated cottonseed oil was a commercial product having also an iodine value of approximately 70. The lard was a steam rendered product purchased on the market.

Young rats from the stock colony were placed upon the experimental diet when 25–28 days of age. The animals were selected so as to have a corresponding litter-mate animal in all groups between which dietary comparisons were made. Two male rats and three female rats composed each experimental group during the first-generation observation. The animals were fed ad libitum and allowed to run on shavings. The only data reported here relating to the reproductive history of the animals, are those given by the number of litters which were born and the number of young which were weaned. No systematic study was made either of the successful matings by the vaginal smear method or of pregnancies as shown by the erythrocyte sign. In some cases, where animals were available, the reproductive ability was observed in succeeding generations.

With the feeding of the above-mentioned experimental diets and the selection of young experimental animals which were fostered by rats receiving the stock ration, initial fertilities and birth of young were not unexpected. Vitamin E and other nutritive deficiencies in the diets could only be indicated in later gestations and succeeding generations after the original body supplies of essential nutrients were exhausted. Emerson and Evans ('39 a, b) reported that increased quantities of vitamin E were required by the female rat as the age increased.

EXPERIMENTAL RESULTS

It will be noted from the data in table 1 that ration A, composed largely of purified foodstuffs and supplemented with additional vitamin E in the form of wheat embryo, was able to maintain reproduction in the fifth generation when hydrogenated cottonseed oil composed 20% of the diet. This was

shown by the fact that each of three females from the fifth generation gave birth to a litter of normal size. When the hydrogenated cottonseed oil in ration A was replaced by an equal quantity of lard and fed to animals of the same breeding and dietary history, the number of young born from first gestations compared favorably with those animals receiving the hydrogenated fat. However, no weaning of the young occurred in any of these groups and it required the second gestation period to demonstrate differences in reproduction and weaning of the young between the lard and hydrogenated-fat groups. One-third as many young were produced from second gestations in the lard group compared to a similar period in the hydrogenated-fat group. As only one animal was weaned on the lard ration, a study of reproduction in the next generation was not possible and the feeding of the lard ration was discontinued. Similar differences were found between two groups of animals receiving lard and hydrogenated cottonseed oil, respectively, in a different kind of diet identified as ration B, which contained no vitamin E added in the form of concentrate, but did contain a supplement of milk and whole liver. Here second generation animals were obtained with each of the fat diets. However, during the observation of the second generation animals for 174 days no young were born to the females on the lard ration while the three females receiving the hydrogenated cottonseed oil diet gave birth to five litters or a total of twenty-eight young.

By using the same type of ration a similar relationship was shown between hydrogenated and unhydrogenated soybean oil in regard to dietary properties as was found in the case of unhydrogenated animal fat (lard) and hydrogenated cottonseed oil. These results are shown in table 2 under ration B. In the group of animals receiving the unhydrogenated oil only one of the three females gave birth to young. This particular female had three litters of young, none of which were weaned. During the same time, four litters or thirty-four young were born to the three females in the group

TABLE 2

Hydrogenated and unhydrogenated fats in rearing successive generations of rats.

| | GENERA-TION | GESTA-TION | NUMBER OF | | | | | |
			Females	Litters	Young	Young removed[1]	Litters weaned[2]	Young weaned
			Ration A [3]					
Hydrogenated	1	1	3	3	15	0	0	0
cottonseed oil	1	2	3	3	27	4	3	14
	2	1	3	2	14	2	2	11
	2	2	3	2	13	2	2	7
	3	1	3	3	21	1	2	12
	4	1	4	4	22	2	2	9
	5	1	3	3	27	7	1	6
Lard	1	1	3	3	14	0	0	0
	1	2	3	3	9	0	1	1
			Ration B [3]					
Hydrogenated	1	1	3	3	18	1	1	5
cottonseed oil	2	1	3	5	28[4]	0	2	12
Lard	1	1	3	3	16	0	3	14
	2	1	3	0[5]	0	0	0	0
Hydrogenated	1	1	3	4	34[4]	3	4	29
soybean oil	2	1	3	4	38[4]	2	4	12
	3	1	3	3	26	0	3	20
Unhydrogenated	1	1	3	3[6]	13	0	0	0
soybean oil	2[7]	1	3	3	20	1	3	16
	3	1	3	1	6	0	0	0
			Ration C [3]					
Hydrogenated	1	1	3	3	19	1	3	18
soybean oil	2	1	3	3	18	3	3	15
Unhydrogenated	1	1	3	3	23	3	3	20
soybean oil	2	1	3	3	17	2	3	15

[1] The maximum number of young allowed to be weaned per litter were six with ration A and eight with rations B and C.

[2] All or part of litter.

[3] Five hundred gram quantities were made at a time and stored in the refrigerator.

[4] Second gestation animals included.

[5] No young at 174 days of age.

[6] From one female, remaining two females had no young during 185 days.

[7] Second generation animals were taken from those which had been weaned on the hydrogenated soybean oil ration.

receiving hydrogenated soy-bean oil; twenty-nine out of the thirty young left to suckle were weaned.

Since no young were available for a second generation study in the unhydrogenated-oil group, young were taken from the hydrogenated-oil group and placed upon the unhydrogenated soybean oil ration. As a result of this procedure young were born and weaned in the group receiving the unhydrogenated oil as well as in the hydrogenated-oil group. Apparently as a result of vitamin E storage from the previous hydrogenated-oil diet and quantities of the vitamin ingested with the milk and liver, the animals were able to function for one gestation period. However, when their offspring were continued on the unhydrogenated-oil ration no animals were weaned in the following generation. Reproduction and successful weaning of the young occurred in the corresponding generation receiving hydrogenated soybean oil.

Diets made up largely of natural food products as reported in table 2 under ration C did not show any difference in nutritive properties which could be attributed to hydrogenation of the soybean oil. This was probably due to certain factors such as the presence of antioxidants in the cottonseed meal and coconut meal and the increased quantities of vitamin E and other nutrients present in the milk powder and the oil meals.

<div align="center">SUMMARY AND CONCLUSIONS</div>

Rats were fed the ordinary sterility ration composed of casein, dextrin, lard, cod liver oil, salt mixture and dried yeast supplemented, in some cases, with wheat embryo and, in other experiments, with small quantities of milk and fresh liver.

In these experiments nutrition was not improved by replacing the lard with unhydrogenated soybean oil. However, when partially hydrogenated soybean oil or partially hydrogenated cottonseed oil replaced the lard, there was a decided improvement in relation to the number of young which were born, the number of young which were weaned, and the breeding of successive generations of rats.

Hydrogenation does not destroy the vitamin E present in vegetable oils. Under conditions where oxidation may cause destruction of essential factors in the diet, use of hydrogenated oil is preferable to the use of unhydrogenated vegetable or animal fats.

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THE AMINO ACIDS REQUIRED FOR GROWTH IN MICE AND THE AVAILABILITY OF THEIR OPTICAL ISOMERS [1]

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THREE FIGURES

The mouse has been used much less than the rat in nutritional investigations, but has proved especially valuable in certain types of cancer studies, a few of which have involved the factor of diet. It would seem desirable, therefore, to know whether the need of the growing mouse for protein can be met by mixtures of purified amino acids suitable for the growing rat (Rose, '38).

The expediency of feeding some of the amino acids in the racemic form justifies determining also whether the mouse can utilize both optical antipodes of an essential amino acid, or only the natural isomer. Although such information is relatively complete for the rat (Rose, '38), few tests of this sort have been made in the mouse. It has been found that $d(+)$-tryptophane and $d(+)$-histidine allow less rapid growth than the natural $l(-)$ forms, and that unnatural $d(-)$-lysine fails to support growth (Totter and Berg, '39). At the outset an additional incentive for making further comparisons in the mouse was presented by reports, since challenged, that tumors

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which arise spontaneously in certain strains of this species contain unnatural amino acids.

The experiments recorded in this communication were directed toward achieving these objectives. They show that a number of amino acids are indispensable to the mouse and they afford qualitative comparisons of the availability for growth of the optical antipodes of methionine, phenylalanine, valine, leucine, isoleucine, and threonine.

<center>EXPERIMENTAL</center>

The mixture of purified amino acids used was patterned after that employed by Rose and Rice ('39) in growth studies on the rat, the chief difference being that the percentage of each amino acid was increased to provide the reputedly greater protein need of the mouse. All of the amino acids were prepared in the laboratory, except the dl-alanine and the $l(+)$-glutamic acid which were commercial products.[2] Nine of the other amino acids were synthesized and nine were isolated from natural sources. Most of the isolation procedures had been used repeatedly in the laboratory, and tests made at various times had shown that if they were applied with reasonable care they were capable of yielding products free of admixed amino acids and other contaminants. The aspartic acid was isolated from the anodic liquors obtained as a by-product in the preparation of arginine, histidine, and lysine. Synthesis of the components of the leucine fraction was chosen to avoid the risk of incomplete fractionation and contamination with methionine encountered in their isolation from natural sources. In several instances synthesis appeared to be the only suitable method of preparation. All of the amino acids were beautiful white products and all had been recrystallized at least once or twice oftener than necessary to show proper melting point, acceptable rotation, and theoretical content of amino or total nitrogen. Composition of the basal amino acid mixture and references to methods of isolation or synthesis, several of which were modified in

[2] Pfanstiehl.

detail, are recorded in table 1. The optical antipodes were obtained by formylating the racemic mixtures and resolving them with brucine. References to specific procedures and data pertaining to optical rotations are presented in table 2.

The basal diet consisted of the amino acid mixture, 33.65; sucrose, 15; starch, 13.35; cod liver oil, 5.0; hydrogenated

TABLE 1

Composition of the amino acid mixture.[1]

	NATURAL AMINO ACID FRACTION	AS FED
	gm.	*gm.*
Glycine (Orten and Hill, '41)	0.15	0.15
dl-Alanine	0.30	0.60
dl-Serine (Schiltz and Carter, '36)	0.15	0.30
dl-Valine (Marvel, '40)	1.50	3.00
dl-Leucine (Marvel, '41 b)	1.95	3.90
dl-Isoleucine (Marvel, '41 a)	1.35	2.70
dl-Norleucine (Marvel and du Vigneaud, '25)	0.60	1.20
l(—)-Cystine (Okabe, '27–'28)	0.70	0.70
dl-Methionine (Barger and Weichselbaum, '34)	0.90	1.80 [1]
l(—)-Proline (Town, '28)	0.30	0.30
l(—)-Hydroxyproline (Klabunde, '31)	0.15	0.15
l(—)-Aspartic acid (Jones and Moeller, '28)	0.30	0.30
l(+)-Glutamic acid	3.00	3.00
dl-Phenylalanine (Marvel, '41 c)	2.25	4.50 [1]
l(—)-Tyrosine (Cox and King, '30)	1.50	1.50
l(+) Arginine, fed as the monohydrochloride (Cox, King and Berg, '29)	0.75	0.90
l(—)-Histidine, fed as the monohydrochloride monohydrate (Cox, King and Berg, '29)	0.75	1.05
l(+)-Lysine, fed as the monohydrochloride (Cox, King and Berg, '29)	2.25	2.80
l(—)-Tryptophane (Cox and King, '30)	0.60	0.60
dl-Threonine (Carter and West, '40)	1.05	2.10
Sodium bicarbonate		2.10
	20.50	33.65

[1] Subsequent changes made in the content of *dl*-methionine and *dl*-phenylalanine in some of the studies are indicated in the text.

TABLE 2

Specific rotation of the isomers used in the feeding tests.

AMINO ACID	$[\alpha]_D$		CONCENTRATION PER 100 CC. OF SOLUTION		SOLVENT USED [1]	TEMP OF SOLUTION READ [1]	REFERENCE
	Found	Recorded in reference	As read	Recorded in reference		°C.	
	degrees	degrees	gm.	gm.			
l(−)-Methionine	− 7.7	− 7.5	0.8	0.8	H_2O	25	Windus and Marvel, '31
d(+)-Methionine	+ 8.12	+ 8.12	0.8	0.8	H_2O	25	Windus and Marvel, '31
l(−)-Phenylalanine	−34.7	−35.14	1.6	1.9	H_2O	20	Fischer and Schoeller, '07
d(+)-Phenylalanine	+33.8	+33.5	2.0	Not recorded	H_2O	26	du Vigneaud and Meyer, '32
l(−)-Leucine	+15.3	+15.8	4.48	4.54	20% HCl soln	20	Fischer and Warburg, '05
d(+)-Leucine	−15.2	−15.5	4.00	4.39	20% HCl soln	20	Fischer and Warburg, '05
l(+)-Valine	+28.0	+28.7	3.20	3.56	20% HCl soln	20	Fischer, '06
d(−)-Valine	−28.22	−28.4	2.80	3.38	20% HCl soln	20	Fischer, '06
l(+)-Isoleucine	+40.03	+40.61	1.60	5.09	20% HCl soln	20	Locquin, '07
d(−)-Isoleucine	−38.3	−40.86	1.60	4.53	20% HCl soln	20	Locquin, '07
d(−)-Threonine [2]	−28.0	−28.3	2.00	Not recorded	H_2O	26	West and Carter, '37
l(+)-Threonine	+28.2	+28.4	2.00	Not recorded	H_2O	26	West and Carter, '37

[1] The solvents and temperatures used in the determinations were those recorded in the reference indicated. In all other instances, the l form is the natural isomer.

[2] d(−)-Threonine is the natural modification.

cottonseed oil,[3] 26.0; salt mixture (Hubbell, Mendel and Wakeman, '37), 5.0; and agar, 2.0%. The vitamin B complex was supplied in the form of two pills daily, each containing 25 mg. of a rice polish concentrate,[4] 0.1 mg. of nicotinic acid, 0.02 mg. of thiamine chloride, 0.02 mg. of riboflavin,[5] corn syrup (50 mg.), and enough starch (usually about 100 mg.) and water to form pellets of suitable size and consistency for molding. The corn syrup lessened the tendency to crumble which obtained when starch alone was added. In a few of the earlier studies gum acacia was used for a similar purpose.

The albino mice were of the Bagg strain.[6] The experimental and control animals were litter mates; usually three or four litters were represented in each test. Individual weights at the start of the experimental period ranged from 8 to 14 gm. Each study was continued for 40 days; weight changes and food consumption were recorded at 4-day intervals. The temperature of the room was thermostatically controlled and care was taken to avoid drafts.

When an optical isomer of any amino acid was tested for its capacity to promote growth, the *dl* form was omitted from the amino acid mixture and the active enantiomorph was incorporated at the natural amino acid level indicated in table 1. Control animals were fed a mixture of amino acids devoid of all forms of the one tested. The starch content of the diet was varied as required to compensate for any change in amino acid content.

The growth data are shown in figures 1 and 2. At the levels fed, $d(+)$- and $l(-)$-methionine were apparently equally effective. This was true also of the antipodes of phenylalanine. In the phenylalanine series the control animals which received neither phenylalanine nor tyrosine lost weight more rapidly than the controls which received tyrosine,

but not phenylalanine. When considered with subsequent tests which show that growth can occur on diets devoid of tyrosine, this observation suggests that the lack of tyrosine imposes a greater need for phenylalanine. Since both isomers of methionine and phenylalanine were effective in promoting growth, only half as much of their *dl* forms was fed in the later studies on isoleucine and threonine.
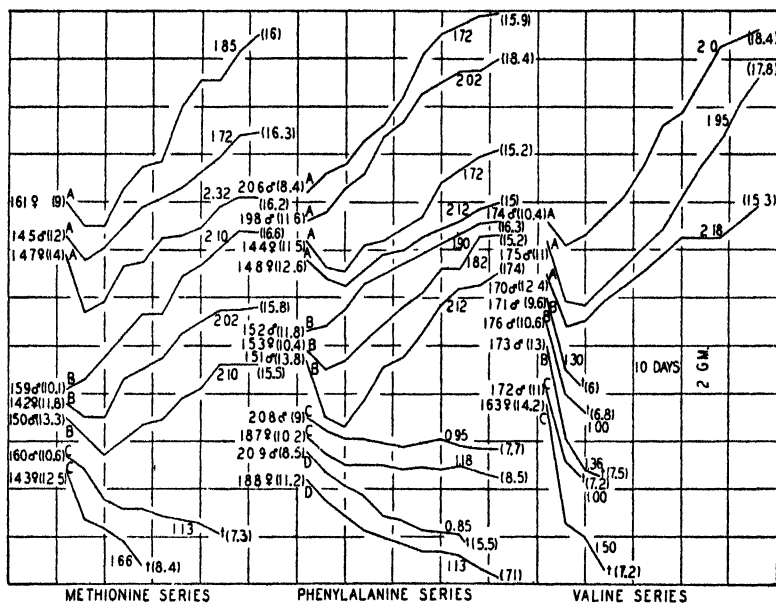
Fig. 1 Comparative rates of growth in mice fed mixtures of purified amino acids containing the natural (A), unnatural (B), or neither (C) antipode of methionine, phenylalanine, or valine. D represents the omission of both phenylalanine and tyrosine. The initial and final weights of the mice are enclosed in parentheses. The daggers indicate death. The unenclosed number which appears near the end of the curve or follows the final weight indicates average daily food consumption in grams.

The tests of the isomers of valine (fig. 1), and of leucine, isoleucine, and threonine (fig. 2) show clearly that only the natural forms of these acids can support growth. In all cases, mice fed the unnatural modifications lost weight as rapidly as control animals fed diets lacking both isomers. Marked

differences in degree of nutritive failure were apparent.
When valine or leucine was omitted, or only the unnatural
isomer of either was fed, the decline in weight was rapid;
marked loss of appetite, extreme weakness, and lack of co-
ordination were noted; and in nearly every instance death
occurred in a few days. Lack of natural threonine induced
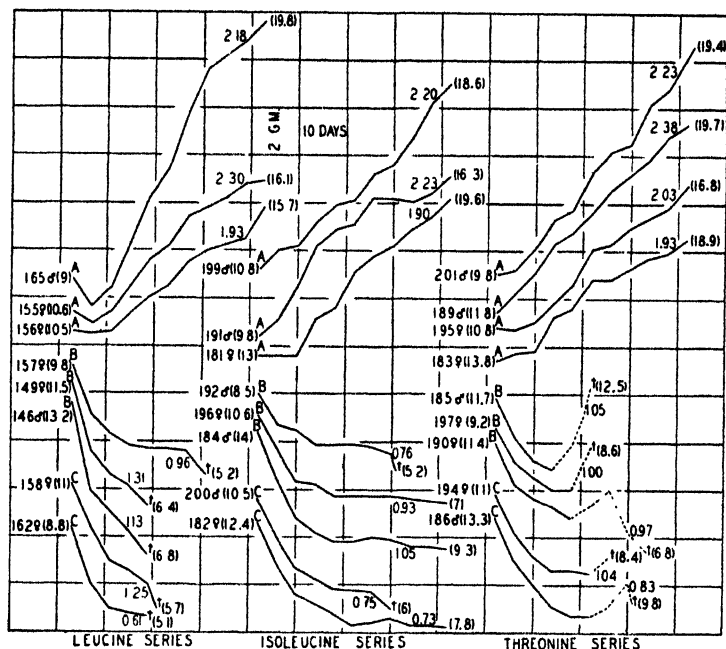
Fig. 2 Comparative rates of growth in mice fed mixtures of purified amino
acids containing the natural (A), unnatural (B), or neither (C) antipode of
leucine, isoleucine, or threonine. The initial and final weights of the mice are
given in parentheses. The daggers indicate death. The broken lines in the
threonine series of curves indicate weight changes associated with the develop-
ment or re-solution of ascites and edema. The average daily food consumption
in grams is indicated by the number near the end of the curve.

a rapid weight loss for 12 to 20 days, followed by a gain. In
the latter interval the animals acquired a general puffy
appearance; abdominal distension was particularly marked.
Three of the five mice died suddenly while still gaining weight;
the other two lost weight again before death. Post mortem

examination showed pronounced edema and ascites in the first three animals. Edema is known to occur in malnutrition (Maver, '20) and has been developed experimentally in rats maintained on low-protein diets (Frisch, Mendel and Peters, '29). Palpable edema had not previously been noted by us, either in mice or in rats fed diets deficient in an essential amino acid; a manifestation so striking as was obtained in this instance could hardly have been overlooked.

The relatively large weight losses of many of the mice during the first 4–8 days on the synthetic diets may be attributed to their failure to accept the diet readily. Mice frequently reject synthetic diets which are potentially able to support growth and which are eaten readily by rats; they seem slower to adapt themselves to dietary changes. Good correlation between growth rate and food consumption was not obtained. This may be attributable in part to differences in growth proclivities; also in part to errors inherent in weighing back scattered food.

Figures 1 and 2 show clearly that methionine, phenylalanine, valine, leucine, isoleucine, and threonine are required by the mouse for growth. Previous studies have indicated the need for tryptophane (Willcock and Hopkins, '06–'07; Wheeler, '13) and histidine (Geiling, '17), and may be interpreted also as suggesting that lysine is necessary. Figure 3 shows that the mouse possesses considerable capacity to synthesize arginine; on mixtures of purified amino acids free of arginine, growth was as rapid as on mixtures containing it, but was not maximal. On a diet which contained 20.2% of commercial casein, 0.3% of cystine, and 8% of yeast three mice of the same strain, weighing 10.6, 11.2 and 11.4 gm. at the outset, gained 14.8, 19.2 and 20.8 gm., respectively, in 40 days, or about twice as much as those fed the amino acid mixture. Whether the capacity of the mouse to synthesize arginine is great enough to allow such rapid growth on an arginine-free diet can be determined only by further study. The cause of the greater growth on the casein-cystine-yeast diet is not of immediate concern, but two possibilities may be mentioned:

(1) The commercial casein contained at least a tenth more total nitrogen than was present in the natural amino acids of the purified amino acid mixture; and the dried yeast contributed additional protein of different composition. Hence, the natural amino acids supplied by the casein-cystine-yeast diet differed in quantity and in distribution from those furnished in the purified amino acid mixture. (2) Casein and yeast also contain considerable phosphorus. Recently Jones and Foster ('42) have presented convincing evidence that the Hubbell, Mendel and Wakeman ('37) salt mixture supplies too little phosphorus for optimum growth in rats, even though fed at levels as high as 4% in diets otherwise low in phosphorus; on the other hand, levels of 2% supported excellent growth when the diet contained other sources of phosphorus, such as casein and yeast. The need of the mouse for minerals is generally considered to be more intensive than for the rat. It is therefore possible that 5% of the salt mixture limited the growth of the mice on the purified amino acid diet which contained no other sources of phosphorus, but was entirely adequate for the mice fed the phosphorus-rich casein and yeast.

In a final test on purified amino acids, mice were fed a mixture of the nine amino acids known to be essential for growth, plus arginine, as the sole source of protein nitrogen. The *dl*-methionine and the *dl*-phenylalanine were fed at levels of 1.8 and 3.4% of the diet, respectively, and the other eight amino acids at levels 50% higher than those indicated in the "as fed" column of table 1. The free amino acids represented 30.3% of the diet and the isomers known to be effective (including both forms of methionine and phenylalanine), 15.6%. The only other dietary changes made were compensatory adjustments in the sodium bicarbonate and starch contents. The curves in figure 3 show that the mice grew slowly on the diet.

In the previous studies of Rose ('38) rats fed these same ten amino acids at an active level of but 11.2% of the diet "gained in weight just as rapidly as when all of the protein components were supplied preformed." The metabolic origins

of the dispensable amino acids which were omitted from the diet are not clearly defined. Growing rats fed diets deficient in cystine produce cystine for tissue synthesis if methionine is available (Beach and White, '39). Apparently tyrosine can be produced metabolically from phenylalanine (Moss and Schoenheimer, '40) and glutamic acid from histidine (Edlbacher and Neber, '34). Perhaps our mice grew at a diminished rate because one or more of the essential amino acids was
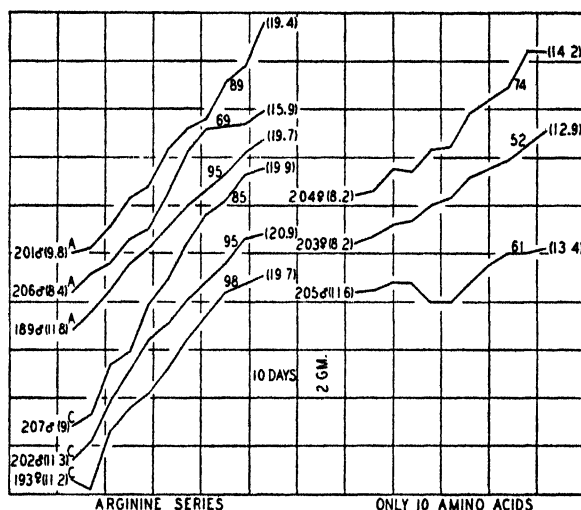
Fig. 3 Comparative rates of growth of mice fed a mixture of twenty purified amino acids (A), a similar mixture lacking arginine (C), or a mixture of only the ten amino acids essential for the rat, including arginine. The initial and final weights are given in parentheses. The total weight of food consumed in grams during the 40-day period is indicated near the end of each curve.

not supplied in an amount sufficiently greater than normal to fully offset its diversion for synthesis of certain dispensable amino acids omitted from the diet. The slower growth could, however, have been caused by some other factor, such as slow rate of synthesis or expenditure of excessive energy in synthesizing so many amino acids simultaneously. In any event, the fact that growth occurred at all suggests that the amino acids which were not fed pre-formed were synthesizable to an appreciable degree.

SUMMARY

Moderate growth was obtained in mice fed mixtures of twenty purified amino acids as the source of protein nitrogen. Both optical forms of methionine and phenylalanine, but only the natural forms of valine, leucine, isoleucine, and threonine, could be utilized for this purpose. The rate of growth was not retarded by the removal of arginine. Evidently, therefore, the mouse is able to synthesize arginine at a fairly rapid rate.

Slow growth occurred when the source of protein nitrogen was limited to the seven amino acids named above and lysine, tryptophane, and histidine. Hence, none of the ten amino acids omitted from the latter mixture could have been absolutely indispensable.

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BIOTIN CONTENT OF MEAT AND MEAT PRODUCTS [1]

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The biotin content of a few meat products has been determined by Cheldelin and Williams ('42) and Lampen, Bahler and Peterson ('42). Since the importance of biotin in nutrition has continued to receive recognition it seemed desirable to have additional information on the distribution of this vitamin in meat and meat products and also to study the retention of biotin during cooking. Several meats were assayed for their biotin content, and the retention of this vitamin was determined during roasting, braising and broiling of pork hams and loins.

EXPERIMENTAL

The Lactobacillus casei method described by Shull, Hutchings and Peterson ('42) and modified by Shull, Miller and Peterson ('43) was used for the biotin determinations.

The biotin was liberated from the meats by treating with 6N H_2SO_4 and autoclaving for 2 hours at 15 pounds pressure. Digestion with pepsin, clarase or pancreatin did not liberate all of the biotin. A representative sample of meat was treated with 2N, 4N, and 6N H_2SO_4 and autoclaved for 2, 4, and 6 hours to determine the best method of hydrolysis in order to give the maximum liberation of biotin. Autoclaving with

4 or 6 normal H_2SO_4 for 2 hours gave maximum liberation, while longer periods of autoclaving resulted in considerable destruction of the biotin. The procedure adopted for liberation of biotin was a 2-hour autoclaving of the sample in 6N H_2SO_4. This procedure was used for all samples analyzed. Satisfactory recoveries of added biotin were obtained when this procedure was used.

After treating the samples with 6N H_2SO_4, the digestion mixture was neutralized with NaOH. It was necessary to determine whether the Na_2SO_4 formed would act as a stimulant or an inhibitor of Lactobacillus casei. The maximum amount of Na_2SO_4 present in any assay tube was 0.7% and it was found that Na_2SO_4 concentrations ranging from 0.2–1.8% had no effect on the assay.

Meat samples 45–257 have been previously analyzed for their vitamin content (Waisman and Elvehjem, '41; McIntire, Schweigert, Henderson and Elvehjem, '43; and Schweigert, McIntire and Elvehjem, '43). Samples 45–168, 258 and 259 were prepared for analysis by removing all visible fat and connective tissue from the outer surface of the meats. These samples were analyzed dry and the millimicrograms of biotin per gram of fresh meat were calculated from the biotin content per gram of dry meat. Samples 170–257 were prepared for analysis by removing the rind and bones from the pork hams and loins. About ½ inch of fat was left on the outer surface. The samples were then passed three times through an electric meat grinder and thoroughly mixed. These samples were analyzed as prepared and the biotin content per gram of dry meat was calculated from the biotin content of the moist meat. The results of the analysis of the meats are found in table 1.

The selection and preparation of the meats used in the cooking experiment have been recently reported (McIntire et al., '43). The loin and hams were cooked without seasoning by standard cooking procedures (Methods of Cooking and Testing Meat for Palatability, '33); the braising procedure has been described by McIntire et al. ('43). In all cases the

TABLE 1

Biotin content of meat samples.

DESCRIPTION OF SAMPLE	SAMPLE NO.	MμG./GM. FRESH	MμG./GM. DRY	DESCRIPTION OF SAMPLE	SAMPLE NO.	MμG./GM. FRESH	MμG./GM. DRY
Kidney				Pork loin			
Pork	62	1348	6235	Fresh	170	56	144
Pork	137	1250	6225	Fresh	180	61	119
Beef	81	923	4050	Fresh	193	47	95
Liver				Fresh [1]	230	43	96
Lamb	96	1270	4375	Braised	183	67	110
Beef	131	966	3315	Braised	196	54	85
Beef	150	1180	4280	Braised [1]	233	49	83
Beef	152	880	2980	Broiled	172	81	162
Fried beef	121	1370	3195	Broiled	182	74	124
Veal	158	752	2820	Broiled	195	61	101
Pork	122	946	2995	Broiled [1]	235	58	105
Pork	153	790	2550	Roast	171	55	115
Pork	159	805	2980	Roast	181	69	134
Heart				Roast	194	58	114
Pork	104	182	815	Roast [1]	231	45	94
Veal	155	150	715	Pan broiled	173	83	153
Beef	133	78	405	Cured hams			
Beef	143	68	358	Smoked	102	96	246
Veal				Cured [1]	251	46	89
Round	103	77	287	Cured [1]	252	61	136
Round	134	63	288	Roast cured [1]	250	59	106
Round	259	38	145	Roast cured [1]	253	83	151
Fried chops	45	190	246	Fried cured [1]	256	100	176
Beef				Fried cured [1]	257	85	155
Round	128	34	144	Lamb			
Round	190	63	251	Breast [1]	243	21	146
Round	258	29	106	Leg	80	59	224
Rib	189	34	124	Miscellaneous			
Pork ham				Beef pancreas	113	137	596
Fresh	175	69	158	Dark chicken			
Fresh	198	44	90	meat	66	101	393
Fresh	185	44	86	Light chicken			
Fresh [1]	236	45	121	meat	68	113	459
Broiled	200	65	112	Beef spleen	76		245
Broiled [1]	239	60	111	Beef lung	138	59	290
Broiled	177	85	175	Beef brain	144	61	288
Broiled	187	75	143	Beef tongue	82	33	119
Pan broiled	178	74	139	Fillet of cod	135	49	287
Pan broiled	201	75	126	Salmon steak	136	59	211
Braised	188	68	126				

[1] Analyzed moist.

drippings were retained for analysis. The moisture and fat determinations and preparation of the meats and drippings for analysis are the same as reported by McIntire et al. ('43), and Schweigert et al. ('43). The detailed results of one of the cooking series are given in table 2.

TABLE 2

Biotin content of meat before and after cooking.

METHOD OF COOKING	BEFORE COOKING			AFTER COOKING					
	Weight of fresh meat	Biotin in meat	Biotin : total in meat	Weight of cooked meat	Biotin of cooked meat	Biotin total in cooked meat	Total biotin in drippings	Retained in cooking	Total retained
	gm.	*mμg./gm.*	*μg.*	*gm.*	*mμg./gm.*	*μg.*	*μg.*	*%*	*%*
Fresh loin, roasted	1105	43	47.6	859	45	38.7	0.61	81	83
Fresh loin, braised	332	43	14.3	242	49	11.9	0.70	83	88
Fresh loin, broiled	347	48	16.7	176	58	10.2	0.32	61	63
Fresh ham, roasted	2348	45	106.0	1542	48	74.2	3.15	70	73
Fresh ham, broiled	801	44	35.2	503	60	30.2	0.55	86	87

TABLE 3

Biotin retention during cooking.

	DETERMINATION	BIOTIN RETAINED IN MEAT ALONE	TOTAL RETAINED
		%	*%*
Roast loin	1	80	82
	2	81	83
Braised loin	1	76	77
	2	83	88
Broiled loin	2	61	63
Roast ham	1	75	76
	2	70	73
Braised ham	1	76	78
Broiled ham	2	86	87

The biotin retained in the meat alone after cooking was calculated by dividing the total micrograms of biotin in the entire piece of cooked meat by the total micrograms in the uncooked meat. The total per cent retained in the meat plus drippings was calculated by adding the micrograms of biotin in the drippings and fresh cooked meat and dividing the sum by the total micrograms in the uncooked meat. The per cent retained in the meat alone and the per cent retained in the meat plus drippings in two cooking series are found in table 3.

DISCUSSION

The biotin content of meat products agrees fairly well with the results reported by Lampen et al. ('42). In most cases our results were higher, particularly in the case of pork kidney, beef kidney and heart. Our results agree very well with the values reported by Cheldelin and Williams ('42), who used Saccharomyces cerevisiae as the test organism. The only discrepancy exists in the biotin content of light chicken meat. They found 54 mμg. of biotin per gram of fresh tissue as compared to 113 mμg. per gram which we obtained. The results obtained with Saccharomyces cerevisiae and Lactobacillus casei methods are, therefore, in very good agreement.

Liver and kidney were found to be the best sources of biotin. Pork kidney averaged 6230 mμg. per gram of dry tissue as compared to 4050 mμg. of biotin per gram for beef kidney. Lamb liver contained 4375 mμg. per gram, beef liver averaged 3530, veal liver 2820, and pork liver averaged 2840 mμg. per gram of dry tissue.

Heart, pancreas, and dark and light chicken meat are good sources of biotin. Beef spleen, lung, brain, and tongue have about the same amount of biotin in millimicrograms per gram of fresh tissue as lamb, beef, veal and pork muscle meats. Pork hams and loins averaged 50 mμg. per gram of fresh tissue.

From 61 to 86% of the biotin was retained in the meat alone after cooking, the lowest retention being observed after broil-

ing pork loin. The total retention of biotin in the meat plus drippings ranges from 63 to 88% of the biotin present in the uncooked meat. The amount of biotin found in the drippings was very small, ranging from 1 to 5% of the total amount of biotin present in the original meat. This indicates that a very small amount of biotin leached out of the meat during cooking in contrast to higher amounts of thiamine, nicotinic acid and riboflavin that were found in the drippings as reported by McIntire et al. ('43) and Schweigert et al. ('43).

The destruction of biotin during cooking is very likely due to the formation of the oxidized form of biotin, which is not utilized by Lactobacillus casei (Nielsen, Shull and Peterson, '42).

SUMMARY

1. Biotin content of various meat products has been determined by the Lactobacillus casei method.

2. Kidney and liver were the richest sources of biotin. Heart, pancreas, and dark and light chicken meat were good sources. Beef spleen, lung, brain, and tongue contain about the same amount of biotin as pork, beef, veal and lamb muscle meats. Pork loins and hams averaged 50 mµg. of biotin per gram of fresh tissue.

3. An average of 77% of the biotin was retained in the meat alone after cooking, and an average of 80% was retained in the meat plus drippings after cooking.

4. From 1–5% of the biotin present in the original meat was recovered in the drippings after cooking.

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THE RETENTION OF VITAMINS IN MEATS DURING STORAGE, CURING AND COOKING [1]

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The retention of vitamins during cooking of fresh pork hams and loins has recently been reported by McIntire, Schweigert, Henderson and Elvehjem ('43). Very little information is available on the retention of vitamins during storage and curing, or cooking of cured meats. Dann and Handler ('42) in preliminary experiments have indicated a definite loss of nicotinic acid on refrigerator storage of meats for 1 week and suggest possible destruction of nicotinic acid on commercial aging of meat. We decided to make a study of thiamine, nicotinic acid and riboflavin retention in pork hams during storage, curing and cooking of the cured hams.

EXPERIMENTAL

Pork hams were taken from carcasses weighing approximately 190 pounds. Paired hams were used throughout the experiment. Two fresh hams were analyzed immediately and the two corresponding hams were stored for 14 days in a freezer ($-4°$C.) in order to determine the vitamin retention during storage.

Two different hams were stored for 14 days and the corresponding two hams were cured commercially in order to study vitamin retention during the curing process. Four

additional hams were cured. Two of these were analyzed un-cooked and the two corresponding hams were used for cooking tests. In all cases the right and left hams were alternated in the duplicate tests.

The hams were cured commercially by placing them in a pickling solution for 12 days. They were then removed, washed with warm water, and placed in a smoke house for 18 hours. At that time the internal temperature of the ham reached 145°F. They were left at that temperature for 2–4 hours, then removed and cooled.

Roasting of the cured hams was carried out under standard conditions without seasoning (Meat and Meat Cookery, '42). Two of the cured hams were roasted in an electric oven at 275°F. until the internal temperature of the meat reached 170°F. The time required for roasting was 7 hours and 50 minutes for one ham and 7 hours and 20 minutes for the other. The drippings from both roasts were retained for analysis.

Two slices ½ inch thick from another cured ham were re-moved parallel to the aitch bone for frying (panbroiling). Similar cuts from the corresponding fresh stored ham were removed for analysis to study over-all vitamin retention from fresh stored to cured fried samples. The frying was carried out as follows: The pan was preheated on an electric stove for 2 minutes and the cured slices were browned on each side at moderate heat for 3 minutes. The rest of the frying was carried out at low heat until the meat appeared well-done. The total cooking time for the ham slices was 16 minutes. The drippings from frying were retained for vitamin analysis.

All hams were prepared for analysis by removing the rind and bones and passing the entire ham three times through an electric meat grinder. The preparation of the drippings for analyses and the moisture and fat determinations were carried out as previously reported (McIntire et al., '43). Vitamin, fat and moisture analyses were run immediately on all samples. The residual solids were calculated by subtracting the per cent of fat and water from 100.

The thiamine contents of the samples and drippings were determined by the method of Hennessy ('42) with modifications of McIntire et al. ('43), the nicotinic acid by the method of Snell and Wright ('41), and the riboflavin method used was that of Snell and Strong ('39) with modifications reported by McIntire et al. ('43).

TABLE 1

Detailed analysis of meat.

DESCRIPTION OF SAMPLE	SAMPLE NO.	PROXIMATE ANALYSIS			THIAMINE		NICOTINIC ACID		RIBOFLAVIN	
		water	fat	residual solids	μg. per gm. fresh	μg. per gm. dry [1]	μg. per gm. fresh	μg. per gm. dry [1]	μg. per gm. fresh	μg. per gm. dry [1]
		%	%	%						
Hog B, fresh	247	49.8	34.0	16.2	6.7	41.3	41	254	2.5	15.4
Hog B, stored	246	52.1	32.1	15.8	5.9	37.3	36	227	2.2	13.9
Hog F, fresh	254	55.1	27.8	17.1	9.5	55.5	42	246	2.6	15.2
Hog F, stored	255	54.8	28.5	16.7	9.0	53.8	40	240	2.1	12.6
Hog C, stored	248	55.8	29.1	15.1	7.8	51.6	36	238	2.0	13.2
Hog C, cured	249	53.5	27.6	18.9	7.2	38.0	38	201	2.3	12.2
Hog D, cured	251	48.2	35.3	16.5	4.7	38.9	21	127	2.0	12.1
Hog D, cured, roast	250	44.7	34.7	20.6	4.7	26.4	26	126	2.0	9.7
Hog E, cured	252	55.2	25.9	18.9	7.2	39.5	34	180	1.9	10.1
Hog E, cured, roast	253	45.0	33.1	21.9	5.0	19.7	36	164	2.1	9.6
Hog C, fresh steak	244	52.5	31.2	16.3	8.5	52.0	37	227	2.1	12.9
Hog C, cured fried steak	256	43.4	24.7	31.9	9.9	31.0	52	163	2.9	9.1
Hog C, fresh steak	245	53.9	29.6	16.5	9.4	57.2	40	242	2.1	12.7
Hog C, cured fried steak	257	45.3	25.0	29.7	9.9	33.3	52	175	2.8	9.4

[1] Calculated on the basis of residual solids.

All of the vitamin analyses were made directly on samples without drying and the vitamin content per gram of dried, ether extracted meat was calculated from these figures. Detailed results are given in table 1. The vitamin content of the meats before and after cooking is summarized in table 2. The per cent of vitamin retention in the meat after storage was

calculated from the vitamin analysis per gram of fresh meat. The per cent retention in the meat after curing was calculated from the vitamin content of the residual solids, although the actual analyses were made on the wet basis.

TABLE 2

Vitamin content of meats before and after cooking.

	CUBED HAM ROASTED	CURED HAM ROASTED	CURED HAM STEAK FRIED	CURED HAM STEAK FRIED
Before cooking				
Sample no.	251	252	249	249
Weight (gm.)	8288	5489	412	479
Thiamine in meat (total mg.)	38.9	39.5	2.97	3.45
Nicotinic acid in meat (total mg.)	174	187	15.6	18.2
Riboflavin in meat (total mg.)	16.5	10.4	.95	1.10
After cooking				
Sample no.	250	253	256	257
Weight (gm.)	5568	3930	248	308
Thiamine in meat (total mg.)	26.1	19.7	2.46	3.05
Thiamine in drippings (total mg.)	5.1	4.3	.11	.25
Nicotinic acid in meat (total mg.)	145	141	12.9	15.7
Nicotinic acid in drippings (total mg.)	31.9	28.0	1.68	1.89
Riboflavin in meat (total mg.)	11.1	8.3	.72	.86
Riboflavin in drippings (total mg.)	1.48	1.15	.09	.10

The per cent retained in the meat alone after cooking was calculated by dividing the total milligrams of the vitamin in the entire piece of cooked meat by the total milligrams in the uncooked meat. The total per cent retained was calculated by adding the milligrams of vitamin in the drippings and fresh cooked meat and dividing the sum by the total milligrams in the uncooked meat.

The over-all retention from storage to cured fried samples was calculated from the vitamin content of the residual solids. The per cent retention after storage, curing and cooking and over-all retention from stored to cured fried samples are shown in table 3.

TABLE 3

Vitamin retention in per cent.

PROCESS	DETER-MINATION	THIAMINE		NICOTINIC ACID		RIBOFLAVIN	
		Retained in meat	Retained in meat plus drippings	Retained in meat	Retained in meat plus drippings	Retained in meat	Retained in meat plus drippings
Storage	1	88		88		88	
	2	95		95		81	
Curing	1	73		84		92	
Over-all retention Fresh stored to cured fried	1	60		72		74	
	2	58		72		74	
Roasting	1	67	80	83	102	67	76
	2	50	61	75	90	80	91
Frying	1	83	87	83	94	76	85
	2	88	96	86	97	78	87

DISCUSSION

The values found for riboflavin and nicotinic acid in fresh hams are about the same as those previously reported (McIntire et al., '43). However, the thiamine content of 6.7 µg. per gram for one of the fresh hams is lower than that for any fresh hams previously analyzed. There is very little, if any, change in water and fat during storage. The residual solids on the cured samples are higher than those on the fresh, indicating loss in water and fat during curing. Both of the fried samples lost considerable water and some fat.

Sample numbers 244, 245, and 248 are all from the same fresh stored ham. The center slices (samples 244 and 245) are higher in thiamine content than the rest of the ham, 8.5 and 9.4 µg. per gram as compared to 7.8 for the remainder of the ham. This would indicate considerable variation in

the vitamin content of different sections of the ham. The nicotinic acid values are more constant, 37 and 40 µg. per gram of ham slice, and 36 µg. per gram for the remainder of the fresh ham. Riboflavin values were still more consistent.

The thiamine, nicotinic acid and riboflavin contents of the fresh and fresh stored hams were similar, although the stored meats showed slightly lower values in all cases.

In the curing process one of the hams was lost; consequently, only one pair of hams could be used for studying retention in curing. Seventy-three per cent of the thiamine, 84% of the nicotinic acid, and 92% of the riboflavin were retained during curing. The values are nearly the same as were observed during cooking, the curing process being actually an extended, low temperature cooking.

Fifty and 67% of the thiamine in the cured ham were retained in the meat alone after roasting, while 83 and 88% of the thiamine were retained in the fried ham steaks. An average of 82% of the nicotinic acid and 75% of the riboflavin was retained in the meat alone after roasting and frying. From 61 to 80% of the thiamine was retained in the meat plus drippings after roasting, and an average of 92% after frying, while 96% of the nicotinic acid and 85% of the riboflavin were retained after roasting and frying. Approximately the same time was required to roast each of the hams, and this may account for the lower retention of thiamine in the smaller ham. The high thiamine retention during frying agrees very well with earlier work (Waisman and Elvehjem, '41). More thiamine was retained after frying than after roasting, braising, or broiling (McIntire et al., '43).

Considerable amounts of each of the vitamins were found in the drippings from roasting and frying, an average of 10% of the thiamine and riboflavin, and 15% of the nicotinic acid.

The over-all retention from fresh stored to cured fried samples agrees very well with retention during curing and frying. Seventy-three per cent of the thiamine was retained during curing and an average of 86% during frying. This

compares with an average of 59% over-all retention as shown in table 3. Eighty-four per cent of the nicotinic acid was retained during curing and an average of 85% after frying, as compared to 72% over-all retention. Similarly 92% of the riboflavin was retained during curing and an average of 77% after frying in comparison to 74% over-all retention.

SUMMARY

1. The thiamine, nicotinic acid and riboflavin contents of fresh, fresh stored, and cured hams before and after cooking were determined.

2. Average retention during storage is 92% for the thiamine and nicotinic acid and 85% for the riboflavin.

3. The retention in curing was found to be 73% for the thiamine, 84% for the nicotinic acid and 92% for the riboflavin.

4. The average retention in the meat alone after roasting was 58% for thiamine, 79% for the nicotinic acid, and 74% for the riboflavin; after frying, 86% for the thiamine, 85% for the nicotinic acid and 77% for the riboflavin.

5. The average total retention in the meat plus drippings after roasting was 70% for thiamine, 96% for the nicotinic acid, and 84% for the riboflavin; after frying, 92% for the thiamine, 96% for the nicotinic acid, and 86% for the riboflavin.

6. The over-all retention of the vitamins from fresh stored to cured fried samples agrees very well with the vitamin retention during curing and frying.

7. From 10–15% of each of the vitamins was found in the drippings from roasting and frying.

8. A higher retention of thiamine in the meat alone was found after frying, as compared to roasting, braising and broiling.

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM IN THE CHICK

I. THE COMPARATIVE EFFECT OF VITAMINS D_2 AND D_3 AND DIHYDROTACHYSTEROL GIVEN ORALLY AND INTRAMUSCULARLY

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It was first noted by Hess and Supplee ('30) that irradiated ergosterol is much less effective than vitamin D from natural sources in promoting normal calcification in the chick. These observations have been amply confirmed, but no satisfactory explanation of the phenomenon has been found. Recently, Correll and Wise ('42) have shown that 1 antirachitic rat unit of dihydrotachysterol is equivalent in the chick to between 4 and 5 units of the U.S.P. Reference Oil. Their results have been confirmed by this laboratory, although we find that the exact ratio of effectiveness varies with the dose level. (A similar relationship of dose level to ratio of effectiveness for the chick has been shown to exist between vitamin D_2 and natural vitamin D by Massengale and Bills ('36).) Our experimental results are given in table 1. They were obtained by the A. O. A. C. vitamin D assay method for chickens with respect to diets and evaluation, except that the vitamin supplements were administered by stomach tube in corn oil, rather than in the diet.

Summarizing previous findings, then, there exists this peculiar situation: Vitamin D_2 is equivalent in the rat to

40,000 U.S.P. units per milligram [1] but in the chicken only to about 1,000 U.S.P. units; [2] vitamin D_8 is equivalent to 40,000 U.S.P. units per milligram in both rat and chicken; and crystalline dihydrotachysterol is equivalent to 80 U.S.P. units per milligram in the rat but to about 360 U.S.P. units in the chicken.

Thus, of two closely related products, both derived from ergosterol, one is more effective antirachitically in the rat than in the chicken, while the other is more effective in the chicken than in the rat. It is perhaps worth calling attention to the fact that equal amounts by weight of vitamin D_2 and dihydrotachysterol have quantitatively rather similar effects in the prevention of rickets in the chicken, while vitamin D_3 behaves in a manner strikingly different from either of these substances.

Beyond mere speculation, the only attempts thus far made to explain the low effectiveness of vitamin D_2 in the chick appear to have been those of Russell and co-workers ('31, '32, '34). They have shown that the amounts of vitamin D_2 and natural vitamin D recoverable from the feces of the chick are quite comparable (26.5 and 34.1% of the dose administered, respectively). It was not definitely settled whether any of the remainder of the dose was destroyed in the digestive tract. However, they showed that cod liver oil was effective in the chick when given either by mouth or intraperitoneally, while six times as many units of vitamin D_2 given intraperitoneally in oil failed to promote equivalent calcification. From these results, they concluded that the low effectiveness of D_2 in the chicken could not be attributed to failure of absorption from, or destruction in, the digestive tract. However, the oils they administered intraperitoneally were not satisfactorily ab-

[1] The most recent authentic information indicates that this value is 40,000 (Coward, '40–'41). However, information at hand in this and other American laboratories indicates that both vitamins D_2 and D_3 may be equivalent to about 50,000 U.S.P. units per milligram.

[2] This value was taken as a convenient approximation. It has been variously reported anywhere from 100 to 2000, but usually from 800 to 1300. For a review of the literature on this point see Remp and Marshall ('38).

sorbed and it is questionable whether the vitamins were utilized quantitatively.

The purpose of the work reported here has been to study comparatively the effects of vitamins D_2 and D_3 and dihydrotachysterol on the calcium and phosphorus metabolism of the chick with the eventual objective of determining so far as possible the reasons for the low effectiveness of D_2. Although

TABLE 1

Antirachitic effect of dihydrotachysterol in the chick.

GROUP NO.	MEDICATION, SUBSTANCE	DOSE U S P. units	DOSE Micrograms	AVERAGE INDEX OF RECALCIFI- CATION [1]	AVERAGE PER CENT BONE ASH	VITAMIN D UNITS (CALCULATED FROM BONE ASH)	VITAMIN D UNITS EQUIVA- LENT TO 1 U.S P. UNIT
1	None	0		—2.0	28.69		
2	Reference oil	1	8696	+0.64	34.26		
3	Reference oil	4	34784	+3.30	44.23		
4	Dihydrotachy- sterol	0.25	3.12	+1.28	37.71	2.05	8.2
5	Dihydrotachy- sterol	0.50	6.25	+2.37	39.31	2.50	5.0
6	Dihydrotachy- sterol	0.75	9.37	+2.90	41.68	3.25	4.3
7	Dihydrotachy- sterol	1.0	12.5	+3.58	45.59	4.75 (approximately)	4.75
8	Dihydrotachy- sterol	2.0	25.0	+3.18	45.02	4.5 (approximately)	2.25
Average							4.9

[1] For method of determination, see McChesney and Homburger ('40).

the response of the chicken to dihydrotachysterol is rather surprising, it cannot be stated whether the effect of this substance in the chicken *or* the rat should be regarded as anomalous until further information is available.

OUTLINE OF TEST METHODS

White Leghorn chicks of both sexes were received in the laboratory on August 26, 1942, on the second day of life. They were immediately offered water and our regular chick ration,

which is slightly modified from that of Hart, Kline and
Keenan ('31); i.e., we substitute two parts corn oil for two
parts yellow corn. From this point on the chicks were managed
as recommended by Massengale and Bills. On the sixth day of
life the birds were divided as to size into eighteen groups
of ten chicks each, at which time the average weight ap-
proximated 62 gm. The depletion period continued until
the seventeenth day of life when medications were begun:
all food consumed was weighed, and the excreta were collected
for weekly periods. Certain groups of chicks were sacrificed
at weekly intervals, as shown in table 2. At the end of the
regular 4-week test period (forty-fifth day of life), all the
remaining groups were sacrificed. Unless otherwise indicated
all the birds in the groups survived the test period.

The birds were killed by decapitation and the blood was
collected in centrifuge tubes from individual animals. The
serum was separated by centrifugation, then pooled samples,
consisting of 1 cc. of serum from each bird in the group, were
analyzed for calcium and phosphorus. Calcium was deter-
mined by the Clark and Collip procedure ('25), and phos-
phorus by the method of Fiske and Subbarow ('25). The left
tibia was removed from each bird and prepared for ash
determination as described by Massengale and Bills.

Food and feces were wet ashed in a mixture of nitric and
sulfuric acids. On the solution thus obtained, calcium was
determined by the procedure of Shohl and Pedley ('22).
Phosphorus was determined by the method of Fiske and Sub-
barow, adjusting the concentration of sulfuric acid so that
the sample taken for analysis was contained in 1 cc. of 2 N
H_2SO_4. The feces, with some spilled food admixed, were
dried, weighed, finely ground in a corn mill, and thoroughly
mixed by shaking. Samples of 2.50 gm. were taken for ashing,
and the appropriate aliquots of the ash solution taken for
analysis. The food was analyzed in the same way and was
found to contain 0.90% calcium and 0.70% phosphorus. During
the test period the chicks received distilled water ad libitum,
changed daily.

Calcium and phosphorus balances were calculated by subtracting the total output in milligrams for the week from the total intake, and dividing by the number of chick-days (i.e., usually 70, but less in case one or more chicks died). Balances are, therefore, recorded in terms of milligrams per chick per day.

Medications were given according to the following plan: oral medications (groups 1 to 12) were given by direct injection by tube into the crop. The vehicle used was corn oil. Quantities of 0.25 cc. were given on the first day of the weekly periods, and quantities of 0.50 cc. were given on the second, fourth and sixth days — or a total weekly volume of 1.75 cc. The intramuscular injections (groups 13 to 17) were given bi-weekly in a volume of 0.1 cc. at each injection. The compounds were dissolved in either corn or sesame oil, or propylene glycol. The injection was made deep into the breast muscle, adjacent to the sternum.

The dosages selected for oral administration were chosen on the basis of the following reasoning. Approximately 3 units of vitamin D_3 per day by the oral route are required to promote entirely normal calcification in the chick over a 28-day period (see McChesney and Homburger, '40). Vitamin D_2 was given in a dosage of 120 units per day on the basis that the effectiveness of D_2 is generally accepted at about one-fortieth that of vitamin D_3. Dihydrotachysterol was administered in a dosage of only 0.6 rat unit per day since this should be equivalent in the chick to about 3 units of D_3, as previously explained (Correll and Wise, '42).

Vitamin D_3 was given intramuscularly in oil at a level of 96 units per week, or about 4.6 times the oral dose. Such a dosage ratio appeared logical, since the observations of Barlow and Kocher ('42) indicated that vitamin A in oil is only 10 to 15% as effective intramuscularly as when the same dose is given by mouth. The work of Liebe ('39) also has shown that in order to produce equal degrees of calcification, the rat requires about ten times as much vitamin D in the form of Vigantol by the parenteral route as is necessary when

given orally in the same medium. Vitamin D_3 in propylene glycol was given intramuscularly at a level of 20 units per week, since Barlow and Kocher have shown that vitamin A in this menstruum is as efficiently utilized whether given intramuscularly or orally. The glycol is miscible with the tissue fluids and therefore does not remain in situ for long periods of time as does oil.

Vitamin D_2 in propylene glycol was also administered intramuscularly in a dosage of 20 units per week in order to compare the effects of an exactly equal number of U.S.P. units of D_2 and D_3 on calcium and phosphorus metabolism when given by this route. Since we can be positive that the solvent used for each form of the vitamin is absorbed, any differences observed in response to this therapy can only be attributed to the specific differences in the manner in which they affect the mineral metabolism of the chick in that any possible difference through destruction and poor absorption from the gastrointestinal tract is ruled out by these procedures. It was for the same purpose that we administered both vitamins D_2 and D_3 intramuscularly in oil, at a level of 96 units per week, thus permitting an exact comparison of these compounds in oil.

Finally, dihydrotachysterol was administered intramuscularly in oil in a dosage equivalent to 22 U.S.P. units per week. This ratio of intramuscular to oral dosage corresponds to that previously selected for the intramuscular oily injections of D_2 and D_3. It was our purpose throughout this experiment to give marginal or preferably suboptimal vitamin dosages since it is under these conditions, rather than with excessive dosages, that comparisons are best made.

RESULTS

The numerical data are given in table 2.

Discussion of results

Growth. The birds treated with the oral supplements of vitamins D_2 and D_3, as well as those which received vitamin

D_3 intramuscularly, grew at a very satisfactory rate. The birds which received dihydrotachysterol showed somewhat retarded growth: those receiving 4.2 rat units (R.U.) per week orally gained only 95% in weight during the 4-week test period as against a gain of 141% for the chicks which received 21 U.S.P. units per week of vitamin D_3. The chicks which received 22 R.U. of dihydrotachysterol intramuscularly showed a still greater retardation of growth in that a body weight of only 60% occurred during the test period. These birds were in a very poor condition and some were unable to survive. This restriction of growth on dihydrotachysterol was also noted by Correll and Wise, although not until they had given oral dosages of the order of 25 units per week (equivalent to about 400 units intramuscularly).[3] They attributed the poor growth to toxicity. It is, of course, possible that the preparation used in this work was more toxic than that used by Correll and Wise. It was not the same one as we used in the test reported in table 1, in which all groups grew satisfactorily. Our laboratory data indicate little difference between the two preparations: they are antirachitically equal in potency and the preparation used in the work reported in table 2 is at most twice as toxic for mice as the one used in the test reported in table 1. For the description of the method used in determining toxicity, see McChesney and Kocher ('42).

The most unexpected response in connection with growth is that of the group which received 96 units of vitamin D_2 intramuscularly per week in corn oil (group 13). These birds were in much worse condition than the negative controls and gained only 41% in weight during the test period. The chickens showed loss of appetite, were extremely weak and unsteady on their feet, and there were many deaths, possibly from malnutrition. It appears that vitamin D_2 given by the intramuscular route in an insufficient dose, that is, 20%

[3] McChesney, E. W., unpublished data.

Effects of various activated sterols on the calcium-phosphorus metabolism of the chick.

GROUP NO.	AGE IN DAYS	AVERAGE WEIGHT, GRAMS	MINERAL BALANCE, MG. PER CHICK PER DAY		BLOOD SERUM MG. PER 100 ML.		BONE ASH PER CENT	VITAMIN SUPPLEMENT PER CHICK PER WEEK
			Calcium	Phosphorus	Calcium	Phosphorus		
1	17	103	53.3 [1]	31.9 [1]				
	24	132			8.4	5.4	35.05	
2	17	105						
	24 [2]	144	38.9	22.8				
	31	182	59.3	45.7	8.6	5.6	35.96	
3	17	107						Vitamin D_3
	24	130	46.1	29.6				in corn oil,
	31	170	70.3	51.6				840 units
	38	204	60.9	42.3	9.0	4.8	38.22	orally
4	17	106						
	24	126	54.0	35.3				
	31	161	52.4	43.1				
	38	215	77.3	51.3				
	45	262	77.4	51.7	9.2	5.3	40.50	
	Group average [3]		60.0	45.0				
5	17	107						
	24	128	44.1	32.0	8.7	5.6	33.95	
6	17	107						
	24	136	47.4	25.6				
	31	169	54.7	36.9	8.9	5.9	37.77	
7	17	111						Vitamin D_3
	24	138	55.4	35.6				in corn oil,
	31	171	43.0	27.7				21 units
	38	235	78.1	51.7	9.3	5.9	39.13	orally
8	17	106						
	24	127	42.6	26.0				
	?1	170	46.7	29.9				
	38	209	86.7	63.7				
	45	255	78.0	51.3	9.2	5.5	39.63	
	Group average [3]		63.5	42.5				
9	17	111						
	24	138	54.1	33.6	7.7	5.2	32.43	
10	17	112						
	24	134	46.4	27.7				
	31	162	36.4	26.1	8.7	5.4	34.48	
11	17	107						Dihydro-
	24	132	49.6	32.0				tachysterol
	31	160	42.0	24.0				in corn oil,
	38	184	44.1	30.9	8.0	5.2	36.79	4.2 units [4]
12	17	108						orally
	24	130	40.4	24.0				
	31	158	43.9	29.9				
	38	183	44.7	23.7				
	45	211	45.3	24.7	8.6	4.9	38.17	
	Group average [3]		44.0	25.5				

TABLE 2 (Continued)

GROUP NO.	AGE IN DAYS	AVERAGE WEIGHT, GRAMS	MINERAL BALANCE, MG. PER CHICK PER DAY		BLOOD SERUM MG. PER 100 ML.		BONE ASH PER CENT	VITAMIN SUPPLEMENT PER CHICK PER WEEK
			Calcium	Phosphorus	Calcium	Phosphorus		
13	17	107	20.6	12.5				Vitamin D₂ in corn oil, 96 units intramuscularly
	24	122	17.8	12.3				
	31	129	15.1	8.7				
	38	139	13.2	10.0				
	45	155 [5]			6.4	6.1	31.42	
		Average	16.8	11.0				
14	17	107	25.9	16.7				Vitamin D₂ in propylene glycol, 20 units intramuscularly
	24	134	17.7	14.7				
	31	159	38.0	28.0				
	38	180	25.4	17.3				
	45	184			6.6	6.0	33.68	
		Average	26.8	19.2				
15	17	106	27.7	21.3				Vitamin D₃ in corn oil, 96 units intramuscularly
	24	139	32.4	29.9				
	31	169 [6]	68.3	44.4				
	38	212	63.0	37.6				
	45	244			9.8	6.3	38.53	
		Average	47.8	33.2				
16	17	106	45.9	28.6				Vitamin D₃ in propylene glycol, 20 units intramuscularly
	24	137	78.4	45.6				
	31	153	108.9	61.6				
	38	220	100.0	62.1				
	45	308			10.0	6.5	45.57	
		Average	83.2	49.5				
17	17	108	27.3	20.0				Dihydrotachysterol in sesame oil,[7] 22 units [4] intramuscularly
	24	124	21.7	18.0				
	31	135	23.4	15.5				
	38	157	23.0	13.1				
	45	173 [8]			9.1	4.7	34.93	
		Average	23.8	16.8				
18	17	103	27.0	21.1				None
	24	121	22.6	15.5				
	31	139	21.9	14.1				
	38	150	19.1	14.3				
	45	158 [9]			7.5	5.2	30.39	
		Average	22.6	16.2				

[1] Balances given are daily averages for the weekly period between the two ages mentioned; i.e., from the 17th to the 24th day of life, and for the period from the 24th to the 31st day of life, etc.

[2] One chick died on the 22nd day of life.

[3] These averages refer only to groups 4, 8 and 12.

[4] In this paper a U.S.P. rat unit refers to the antirachitic effect of 1 unit of U.S.P. reference oil in the albino rat. In terms of the crystalline preparations referred to it would be: vitamins D₂ and D₃, 0.025 μg.; dihydrotachysterol, 12.5 μg. In the chick a U.S.P. unit would be approximately: vitamin D₂, 1 μg.; vitamin D₃, 0.025 μg.; dihydrotachysterol, 2.8 μg.

[5] Two chicks died on the 23rd day of life, one on the 35th, one on the 39th, two on the 43rd and one on the 45th.

[6] One chick "missing" on the 28th day of life; presumably died.

[7] Sold by the Winthrop Chemical Company under the trade name, "Hytakerol." One ml. contains 1.25–1.50 mg. dihydrotachysterol and assays 100–120 U.S.P. rat units.

[8] One chick died on the 33rd day of life, one on the 36th, and one on the 43rd.

[9] One chick died on the 44th day of life.

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or less of the curative dose, weakens rather than strengthens the chicks. Further study will be required to account for this reaction. Those chicks which received 20 U.S.P. units of vitamin D_2 weekly on propylene glycol also did not grow very well, but they grew better than the negative controls, and as well as the birds which received dihydrotachysterol by mouth.

Mineral balances. The results indicate that the amount of mineral retention does not necessarily increase with each week of life and that under oral dihydrotachysterol medication the retentions are practically uniform for each week as contrasted with vitamins D_2 and D_3 where they generally increase each week. The effects of the last two named were quite comparable, and the response to medication was immediate.

The birds which received vitamin D_2 intramuscularly in oil showed balances much poorer than the negative controls. However, those which received the vitamin intramuscularly in propylene glycol showed balances slightly greater than those of the negative controls. The data indicate further that vitamin D_3 given intramuscularly in oil produces no immediate effect on calcification. By the third week the birds began to retain amounts of calcium and phosphorus almost equal to those observed in the animals which received the vitamin orally. The results of the analyses indicate that the vitamin is very slowly or poorly utilized when administered in oil by the intramuscular route. The birds which received the vitamin D_3 intramuscularly in propylene glycol made the most satisfactory showing of all the groups tested. The birds which received dihydrotachysterol intramuscularly in oil had mineral balances almost exactly the same as those of the negative controls.

Blood levels. Although no control figures were obtained for the birds at the start of the test period, it is apparent that the values for calcium at least were low. The most prompt recovery was noted in the birds which received vitamin D_3 orally, the values for both calcium and phosphorus being only slightly below normal at the first observation, and improving

steadily thereafter. The changes in phosphorus from week to week hardly seem significant as no trend is evident. The birds which received vitamin D_2 orally showed improvement in calcium levels for each week, but the values obtained were always slightly lower than those in the D_3 chicks until the last week. There is no apparent reason for the low phosphorus value for the third week.

The chicks which received dihydrotachysterol orally gave particularly low calcium levels for the first and third weeks and the values were, in fact, always at a lower level than in the D_2 or D_3 birds. However, the phosphorus values for these birds did not differ significantly from those of the other groups mentioned.

As for the blood levels of the birds which received the intramuscular injections, those which received vitamin D_3 in either solvent showed very satisfactory blood levels, indicating an entirely normal state of calcium and phosphorus metabolism at the end of the test period. The negative controls showed low calcium, but normal phosphorus values. The birds which received vitamin D_2 in either solvent showed deranged metabolism as indicated by very low serum calcium. The values obtained for the birds which received dihydrotachysterol were at the lower limits of normal.

Bone ash. The bone ash percentages are somewhat lower than those obtained in the usual chick D assay, because of the rather long depletion period. Nevertheless, the oral vitamin supplements were sufficient in every case to permit the birds to show an increased ash value for each week of the test period.

The bone ash values in general agree with what would have been expected from the observed mineral balances. There are some exceptions to this, notably in group 9 where a rather high balance is accompanied by a low ash. Otherwise, with respect to groups killed at the same time there is a good correlation between mineral balances and bone ash.

Relative effectiveness of preparations. The results obtained make possible some tentative conclusions as to the relative effectiveness of the various preparations tested. Since vita-

min D_2 was administered orally at a level of 40 units for each unit of vitamin D_3, and gave a slightly superior result, it is evident that in these experiments the previously reported ratio of effectiveness of about 35 to 1 is confirmed. Dihydrotachysterol was administered orally at a level of 1 unit for each 5 units of vitamin D_3 and it gave a slightly inferior result (as regards bone ash); therefore in these experiments and at this particular dose level, the relative effectiveness must have been approximately 4 units of D_3 equals 1 unit of dihydrotachysterol. Because of the lack of points on the dose-response curve, a more exact quantitative comparison is not possible, but this will be the subject of a subsequent publication.

Vitamin D_2, administered intramuscularly in either oil or propylene glycol in the same unit doses as vitamin D_3, exerted a much inferior antirachitic effect. Quantitative comparison of the two vitamins is not possible. The same applies to dihydrotachysterol in oil. However, the difference between groups 15 and 17 indicates plainly that dihydrotachysterol in oil by muscle is considerably less than four times as effective as vitamin D_3 per rat unit by the same route.

Vitamin D_3 given at a level of 20 units per week in propylene glycol intramuscularly is much more effective than 21 units orally in oil, indicating that there is a serious loss in absorption (probably at least 50%) and that by the intramuscular route the vitamin must be as completely utilized as can be accomplished by any route of administration.

CONCLUSIONS

1. The ratios of oral effectiveness in the chick of $D_3 : D_2 =$ 35 : 1, and of dihydrotachysterol : $D_3 =$ about 4.5 : 1 (rat unit for rat unit) have been confirmed.

2. Vitamin D_2, given intramuscularly in either oil or propylene glycol, and at the same unit dose level as vitamin D_3, has a much inferior effect and the mineral balances of the chicks are very low.

3. Dihydrotachysterol administered intramuscularly in oil is not as effective relative to vitamin D_3 as when the two preparations are administered orally.

4. Vitamins D_2 and D_3, administered orally in doses equivalent in antirachitic effect (for the chick), maintained normal calcium and phosphorus metabolism. However, a dosage of dihydrotachysterol equivalent in antirachitic effect to these does not serve to maintain normal serum calcium levels and the mineral retentions are less.

5. Vitamin D_2 and dihydrotachysterol, administered intramuscularly in small doses in oil, definitely weaken the chicks.

6. Vitamin D_3 administered intramuscularly in propylene glycol is much better utilized than when given orally in oil. When given intramuscularly in oil it is much more poorly utilized than when given orally.

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VITAMIN K AND PROTHROMBIN LEVELS WITH SPECIAL REFERENCE TO THE INFLUENCE OF AGE [1]

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It is now firmly established that vitamin K is concerned in the production of prothrombin, but the mode of its action remains obscure. Investigation of this mechanism from the metabolic standpoint immediately raises the problem of exact quantitative relationship between the vitamin K intake and the prothrombin level, and of factors which may influence this quantitative relationship. Among the more obvious of such factors are the age and rate of growth of the animal, tissue storage of the vitamin and of prothrombin, and the functional integrity of the tissues concerned in the production and utilization of prothrombin.

A certain amount of more or less incidental information of a quantitative nature has been obtained in connection with various assay procedures for vitamin K, but unfortunately, most of the assay procedures have been based simply upon hemorrhagic symptoms, or upon crude clotting time tests. The data are therefore not quantitative with respect to prothrombin levels. In the clinical studies on man, more careful attention has been given to the prothrombin level, but the patient's welfare usually has dictated the use of large amounts

of the vitamin, and very little is known about the response to suboptimal dosage.

Of the data available, perhaps the most significant from the metabolic standpoint is some of the vitamin K assay work of Almquist, Mecchi and Klose ('38), and of Almquist and Klose ('39). These investigators reported that in the chick the reciprocal of the clotting time or the prothrombin time approximates a straight line function of the logarithm of the vitamin K level in the diet. The relationship is purely empiric, and is not reliable for minor degrees of deficiency. Their work was carried out with vitamin K concentrates rather than with some of the pure synthetic preparations now available, and the prothrombin level was determined for only a single interval for each individual chick. Furthermore, the active principle was given ad libitum, by adding it directly to the diet, and there is no precise way of knowing either how much was eaten, or how much may have been lost by deterioration. It is particularly difficult to compare the amount of vitamin actually consumed by chicks of different ages when the vitamin is administered in this way. It was therefore thought desirable to establish more exact baselines for metabolic work, with elimination of these various uncertainties, in so far as possible. In the work reported at this time we have employed the two-stage prothrombin method which, in our experience, is more precise than the one-stage procedures for estimating the prothrombin level. Also, the one-stage tests do not show a difference between the prothrombin of the young and of the adult, whereas with the two-stage method, the prothrombin of the chick, like that of other species, is shown to be low normally at hatching and to gradually rise to the adult level over a period of several months (Tidrick, Joyce and Smith, '39).

EXPERIMENTAL

Newly-hatched White Leghorn chicks were placed on a slightly modified Almquist's diet, as described in a previous publication (Tidrick, Joyce and Smith, '39). The animals were kept on raised wire screens to minimize coprophagy and a

constant source of running water was provided to prevent contamination of the drinking water. Blood for prothrombin determination was drawn into potassium oxalate solution from the heart or from the jugular vein. The blood was centrifugalized, and the assays made on the plasma by the two-stage method of Warner, Brinkhous and Smith ('36), except that chick brain instead of beef lung was used as the source of thromboplastin. The daily dose of vitamin K (2-methyl-1, 4-naphthoquinone), dissolved in 0.2 cc. of corn oil [2], was introduced directly into the crop with the aid of a syringe and metal tube.

The normal control group of 198 chicks received a diet which was rich in natural vitamin K, but, to ensure an abundance of the vitamin, each chick was given a supplement of 12.5 µg. of the synthetic vitamin daily by injection directly into the crop. The test animals, approximately 300 chicks, were placed on the deficient diet and given varying doses of the vitamin. They were about equally divided among the various vitamin K dosage levels. A part of them were given the vitamin supplements from the time of hatching; the remainder were given no vitamin for the first 10 days of life and were then given restorative doses varying in size. By the age of 2 weeks, the prothrombin level had become stabilized for each vitamin K dosage and the chicks given no vitamin for the first 10 days had reached essentially the same levels as those given the vitamin supplements from the time of hatching.

RESULTS AND DISCUSSION

In figure 1, the data are plotted to show the relation between the vitamin K intake and the prothrombin level after stabilization had been reached. The average prothrombin level for each dosage at the age of 17 days is plotted in the solid columns. It is evident that as maintenance requirements were approached a given increment in the amount of vitamin administered had somewhat less effect. Amounts greater than 2 µg. per day did not produce any further elevation of the

[2] Mazola.

prothrombin level and the shape of the interrupted curve would indicate that the minimal requirement cannot be much less than this amount.

In the continuous curve, we have plotted hypothetical prothrombin values, obtained by calculating the prothrombin level as a simple function of the logarithm of the vitamin K intake, as suggested by the work of Almquist et al., mentioned above. With large doses of the vitamin, i.e., larger than 2 µg., the logarithmic relation could not be expected to hold; they were
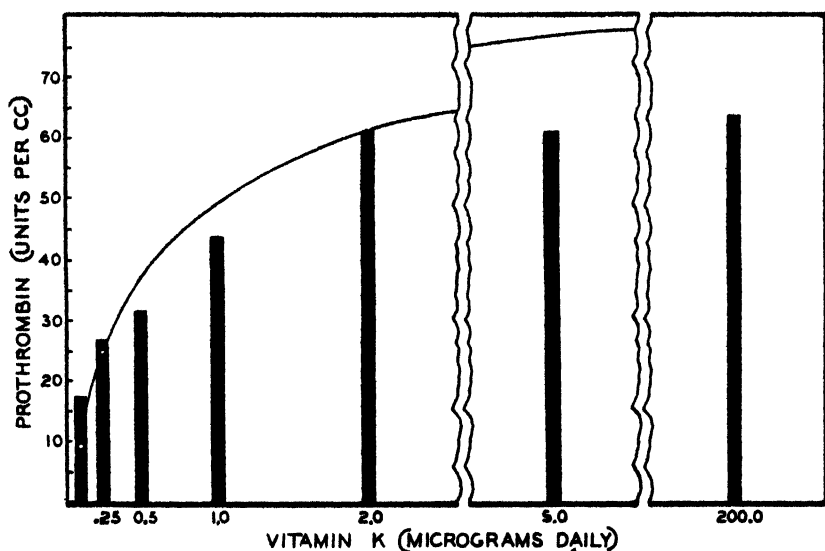
Fig. 1   Prothrombin levels with various vitamin K dosages.

therefore not given consideration in determining the constants for the logarithmic equation. Also, the lowermost observation (0.1 µg.) deviates rather markedly from the hypothetical curve and this value was given less than its full weight in determining the constants which gave the best fit with the experimental data. It may be that the prothrombin level obtained with 0.1 µg. per day is too high. Minor degrees of contamination as a result of coprophagy, or residual vitamin in the diet, would be expected to result in a conspicuous error at this low dosage level. Even with these limitations, it can be seen that

the approximation of the hypothetical values to the actual prothrombin levels obtained experimentally is not very exact. It is of interest that the animals maintained on the extracted diet did not attain quite as high prothrombin values as did the normal controls, even though massive doses of the vitamin were given. Thus, those given 200 μg. per day reached a level of 62 units whereas the control group, as shown below, reached a level of 68 units per cubic centimeter at this same age. This difference might be interpreted to mean that the synthetic
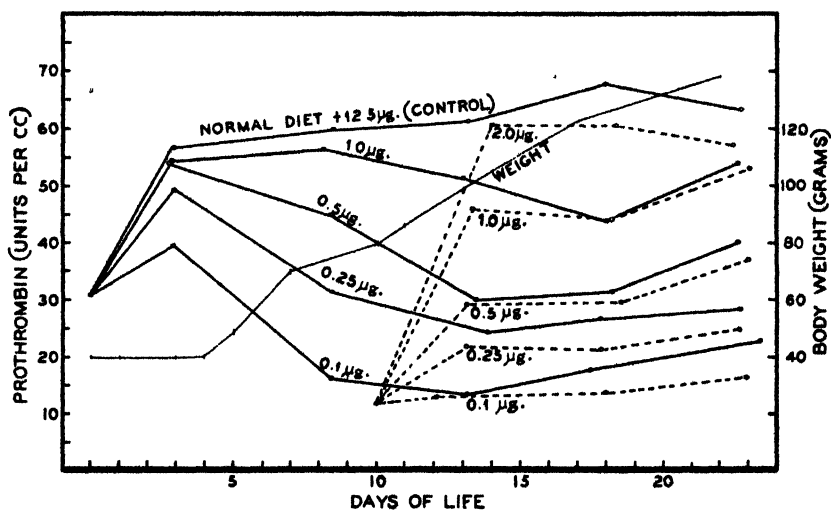
Fig. 2 Prothrombin levels with increasing age.

material was not as effective as the natural vitamin K present in the unextracted diet. It seems more likely, however, that the normal control diet (brewers' yeast 8.0%, fish meal 17.0%, polished rice 61.5%, alfalfa meal 10.0%, salt mixture 2.5% and cod liver oil 1.0%) contains some other essential factor, and that a deficiency of this factor interferes with completely normal prothrombin production.

In figure 2, the data are plotted to show the influence of age and size of the chick on the prothrombin level with varying amounts of vitamin K. The animals receiving the vitamin from the time of hatching are indicated by the continuous

curves; those given no vitamin for the first 10 days, by the broken lines. For simplicity in charting, the experimental period was divided into 5-day intervals, the average pro- thrombin level and the average age of the chicks when tested being determined for each 5-day period.

During the first few days of life the prothrombin level rose rapidly above the value at hatching of approximately 31 units per cubic centimeter of plasma. The rise was greatest in the normal control animals given an enriched diet with a supplement of 12.5 µg. of the vitamin per day. The low hatching level shown in the chart does not appear, however, to be due to a shortage of vitamin K, for similar values have been ob- tained even though large amounts of the synthetic vitamin were administered to the laying hens (unpublished data). Also, by injection of the vitamin directly into the incubating eggs, the newly-hatched chicks can be shown to have excess stores of the vitamin (Tidrick, Stamler, Joyce and Warner, '41), and despite the presence of such stores, the hatching level remains low. Furthermore, there was a similar initial rise in prothrombin in the chicks given minimal amounts of the vita- min. Even in the chicks given no vitamin whatever (not shown in the figure) there was a definite rise in prothrombin level during the first few days. This initial rise may be due to increased activity of tissues which are becoming more mature. On the other hand, it seems equally possible that the hatching level is pathologically low as a result of some deficiency other than vitamin K, and that this deficiency is rapidly corrected when the chick begins to eat. Some such hypothetical deficiency is suggested by the fact that we occasionally obtain a batch of eggs in which the hatching level is as high as 50 units per cubic centimeter. This would seem to imply that the hatching level of 31 units per cubic centimeter shown in the figure is not necessarily normal. In our experience, eggs with an un- usually high hatching level are more often obtained in the summer and early fall months, when a widely varied diet is available to the hens.

After the initial rise, the prothrombin becomes more or less stabilized at a level determined by the amount of vitamin K administered. With the lower dosages of the vitamin, this stabilization was at a level much lower than that present at the time of hatching. The chicks given no vitamin for the first 10 days of life were started on supplements of the various dosages at this time in order to approach the prothrombin level attained for each dose from the opposite direction. By the age of 15 days these animals, shown in the broken lines on the chart, reached essentially the same levels as obtained in the animals given the vitamin from the time of hatching, though there was a slight tendency to lag on the part of the animals depleted for the first 10 days.

In all of this work there was considerable variation in the prothrombin values from chick to chick, with some actual overlapping for the different dosage levels. The differences between the average prothrombin levels for each group, however, are consistent throughout the experiment, and at most points exceed the probable error of the difference severalfold. At the age of 17 days, for example, the observed difference in the average prothrombin values for the 0.5 µg. and the 1.0 µg. groups receiving the vitamin from the time of hatching, is three and one-third times the probable error of that difference.

Following stabilization, the prothrombin level for each dosage tended to rise gradually throughout the remainder of the experiment, despite the fact that the vitamin was kept constant. Even in the groups given only 0.1 µg. per day — an amount barely sufficient to prevent death from hemorrhage during the first 2 weeks of life — there was a definite tendency for the prothrombin level to rise slightly as the chick grew older. Data for older chicks are not as complete as for the age period shown in the chart. We have, however, obtained sufficient data to indicate that the prothrombin level continues to rise slowly during the next 2 months. This is somewhat surprising when one considers that the chick at 3 weeks of age has trebled in weight, and at the end of 2 months weighs nearly fifteen times the hatching weight.

It is of course possible that traces of vitamin K remained in the extracted diet and that, with increasing food intake, the older chicks obtained increasing amounts of this contaminating vitamin. Both the brewers' yeast and the fish meal used in the diet, however, were subjected to continuous ether extraction for 10 days in a giant Soxhlet apparatus. The cod liver oil was used in only small amounts (1%) and no vitamin K could be demonstrated in the preparation used by giving large amounts of the oil to test chicks. Thus, it seems unlikely that appreciable amounts of vitamin K could have remained in the diet. As a second possible source of contamination, greater production of vitamin K by bacteria in the intestine could conceivably supply additional vitamin as the chicks increased in age. That these are not important sources of vitamin K in these experiments, however, is indicated by the fact that minute increments in the vitamin K supplement continued to have a marked effect on the prothrombin level. Were the chicks obtaining significant amounts of the vitamin from incomplete extraction of the diet, or from bacterial synthesis in the intestine, one would hardly expect the groups on slightly different dosage levels to continue to have significantly different prothrombin levels.

It is evident that the vitamin K requirement of the chick is not related in any simple way to the body weight, or surface area. The requirement seems more nearly analogous to the decreasing need for pantothenic acid in the growing rat (Unna and Richards, '42), or to the iron requirement of the growing child (Heath and Patek, '37). The lack of need for additional vitamin K as the animal increases in size might be explained on the basis of progressively greater efficiency of utilization. On the other hand, it may well be that the amount of vitamin required for maintenance is minimal. If so, one might postulate that the vitamin K which is required is needed mainly for growth. There is very little evidence, however, that the vitamin is needed for increase in size itself, for the animals grow at the normal rate even when a severe deficiency exists. The need of the growing organism for relatively large rations of

vitamin K may represent merely the needs for building new prothrombin to correspond to the increase in body size and blood volume.

SUMMARY

Young White Leghorn chicks require 1.0 to 2.0 µg. of 2-methyl-1, 4-naphthoquinone per day to maintain the prothrombin at a normal level. Only about one-twentieth of this amount, however, is necessary to maintain sufficient prothrombin in the blood to protect the animal from hemorrhagic manifestations. Thus, there is a wide range between the amount of vitamin which will support normal prothrombin levels and the amount necessary for protection from hemorrhage.

There is no increase in the amount of vitamin K needed by the young chick as the animal increases in age, despite the fact that the body weight increases severalfold within a few weeks. The data suggest that a large portion of the vitamin needed by the growing animal is associated with the increase in size.

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EFFECT OF SEVERE CALCIUM DEFICIENCY ON PREGNANCY AND LACTATION IN THE RAT [1]

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In a previous study (Boelter and Greenberg, '41) it was reported that growing rats reared on a severely calcium deficient ration showed (a) retardation of growth, (b) decreased food consumption, (c) raised basal metabolic rate, (d) reduced activity and sensitivity, (e) extreme demineralization of the skeletal structures, (f) a high death rate, and (g) susceptibility to internal hemorrhages resulting in collapse, prostation and paralysis of the hind limbs.

In the course of this work it was noted that rats which had been maintained from the time of weaning on a diet deficient in calcium failed to mate, although there was no apparent gross or histological change in the reproductive organs. Pregnancy and, more particularly, lactation cause a heavy drain on the calcium and phosphorus stores of the maternal body. This was true in the rat, as has been shown by Goss and Schmidt ('30), even when the diet contained a normal level of calcium and phosphorus. The above considerations led to the study of the effects of severe dietary calcium deprivation on the female animal and its young during gestation and lactation periods.

EXPERIMENTAL METHODS

Twenty-six mature female rats, each of which had borne at least one litter, were transferred from the stock colony ration to the low calcium diet for the purpose of this investigation.

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The diets used were similar in composition to those employed and reported in detail in the previous study of the effect of low dietary calcium on growing rats (Kleiber, Boelter and Greenberg, '40), with the exception of the addition to the food of 1% wheat germ oil. The calcium deficient diet contained 10.6 mg. calcium, 85 mg. magnesium and 425 mg. phosphorus per 100 gm. food; the control diet, which was identical but supplied with adequate calcium, contained 546 mg. calcium, 84 mg. magnesium and 436 mg. phosphorus per 100 gm. food. The same analytical methods were used as in the prior investigations (Boelter and Greenberg, '41).

<div align="center">RESULTS</div>

1. Reproductive success

Production of young from the twenty-six animals, after forty-eight attempts at mating, was noted in only fourteen cases. Only six mothers gave birth to living young. The number of viable young noted in each of these six instances was 1, 2, 3, 7, 7 and 8, respectively. The majority of the remaining mothers ate the young which were dead at birth or which died soon after birth. Six to eight young per litter occurred most commonly. It is possible, where only one to three baby rats were found, that the rest of the litter was eaten by the mother. This conclusion is supported by the fact that the mother's loss in body weight at parturition in each case, no matter what the size of the litter, corresponded approximately to the weight of a litter of eight.

In the majority of cases, two attempts at mating the calcium deficient rats were made. Only one mother was shown definitely to give birth to a litter both times. Her first litter was dead at birth and eaten by her. The second litter consisted of seven living young. Pregnancy doubtless was initiated in other females in the two trials but was not carried through the complete gestational period in both instances. In general, the second attempt at mating was more successful than the first, not only in the number of litters obtained from the group of

rats as a whole, but also in the number of living young obtained per litter. From this fact it is evident that the length of time the rats were maintained on the low Ca diet did not affect materially the reproductive success.

Immature rats, weighing less than 100 gm. at the time they were changed from the stock diet to the calcium deficient diet, after reaching adult age invariably failed to mate on repeated trials. Such rats had an abnormal estrous cycle, indicated by the fact that the vaginal smears showed a continuous diestrous stage rather than the usual cyclical changes. This was evidence of the failure of the deficient animals to gain sexual maturity on reaching adult age. The failure to mate appeared to be the result of actual changes in the reproductive capacity of the rats related to the dietary lack of calcium, but it may have been due simply to the effects of inanition and the general apathy of these animals. Adult female rats transferred to the calcium-inadequate diet after they had reached maturity showed a normal estrous cycle.

Litters of six, eight and ten rats, respectively, were produced by three animals maintained on the control calcium-supplied synthetic diet since the time of weaning. The young were smaller in size than those of the stock colony. The synthetic rations, although they allowed for reproduction and lactation, were not nutritionally optimal. The difference between the stock colony and control young will be pointed out in the ensuing paragraphs.

In 1924 Simmonds observed that rats were able successfully to suckle two litters of young when placed on a diet inadequate in calcium (0.103%) but they subsequently failed to reproduce. Bodansky and Duff ('41), in a study of the effect of various dietary regimes upon the course of parathyroidectomy of the rat, noted that mating of rats was unsuccessful on a low calcium diet (0.017%). Among the different rations employed, the most marked reduction in fertility occurred with the diet lacking in calcium.

2. Symptoms

Even though the rats were deprived of calcium after they were no longer growing, if they were maintained on the inadequate rations long enough they suffered the same consequences shown by young growing rats as the result of calcium deficiency (Boelter and Greenberg, '41). The time of appearance of the symptoms was delayed and the severity lessened for the mature rats. Typical spontaneous paralysis was noted after 12 weeks of calcium deprivation, although severe fatal paralysis did not occur in a group of six female rats for 21 to 35 weeks. Only one death occurred during the gestation period. There were no deaths prior to gestation or during and immediately following lactation.

Convulsive tetany was never observed and could not be induced in either the calcium-deficient mothers or their young.

3. Changes in body weight

Mature female rats failed to maintain their body weight after they were transferred to the low calcium diet. The magnitude of the weight change was slight until the rats had been on the deprivation regime for a long period of time. There was little difference between the weight changes observed among the rats which gave birth to litters and those which did not bear any young. Calcium-supplied control animals maintained their weight after reaching maturity even if subjected to pregnancy and lactation.

The greatest loss in weight occurred at the time of the appearance of spontaneous paralysis in some members of the group. This time also coincided with the period immediately following pregnancy. The demands of pregnancy were probably an additional strain upon the animal causing a loss in body weight and making the rat more susceptible to the consequences of extreme calcium deficiency. A fairly constant body weight was maintained after the initial drop for as long as 40 weeks, unless the rats became paralyzed and prostrate.

Both calcium-deprived rats and their calcium-supplied controls behaved in the usual way with respect to body weight changes as the result of pregnancy. The weight gained during gestation equalled aproximately that lost following birth of the young. The calcium-deficient animals gained an average of 50 gm. during the gestation period and lost 45 gm. at parturition. The controls gained similarly 57 gm. and lost 45 gm. Calcium-deficient mothers which lost weight during the gestation period gave birth to non-living young. The three control females were younger than the deficient ones at the onset of pregnancy, which, furthermore, was the first for the control mothers. The latter animals, in contrast to the deficient rats, had been reared on the synthetic rations from the time of weaning.

Only three calcium-deprived mother rats were maintained on the low calcium diet during the lactation period, and only three young per mother were allowed to survive the whole 28-day interval. During this time each mother lost approximately another 50 gm. body weight. On the other hand, three calcium-supplied control mothers were able to maintain their body weight while nursing three to five young each, and they showed a small gain in weight following the lactation period, as they had not yet reached their maximum weight. It is probable that the calcium-deprived mothers would not have been able to care for the complete litter for the whole lactation period, and certainly the mothers would have lost a greater amount of body weight, if most of the young had not been sacrificed.

The average weight of all the young at birth was normal — about 5.0 gm. At weaning time (28 days) the average weight of the young was as follows: calcium deficient, only 23 gm.; control young, 37 gm.; and stock colony young from a litter of eight rats, 50–60 gm. apiece.

Those calcium-deficient young which were not sacrificed during the lactation period for analytical purposes were maintained on the low calcium diet after weaning. They gained, on the average, 5 gm. the first week and 19 gm. the second

week. After this they lost weight (18 gm.) and most of them died within 2 to 4 weeks after weaning. Control young were maintained on the calcium-supplied synthetic diet for as long as 20 weeks after weaning, during which time they were normal in every respect, except that their growth rate was below that of the stock colony young. The calcium-deficient young showed no symptoms of abnormality, except their minute size, until they suffered induced paralysis. The galvanic stimulus produced instant collapse and paralysis within 10 days of weaning, followed within a few days by the death of the animal. Autopsy showed a typical calcium deficient hemorrhagic condition (Boelter and Greenberg, '41).

The small size of the calcium-deficient young at weaning probably is due to their partial starvation as the result of the poor milk supply of the low calcium mothers. Apparently the calcium deficiency of the mother does not affect the development of the young in utero, but it does hinder materially the growth of the infant rats after birth. The death of many of the young rats soon after birth is to be attributed to the failure and inability of the calcium-deprived mother to care for them.

4. Food consumption

The first few weeks after being transferred to the experimental rations, the mothers consumed only 4–6 gm. of the calcium-low food per rat per day, which is the usual amount for a rat on such a dietary regime. However, the food consumption rose subsequently to normal (9–11 gm. per rat per day) and during pregnancy and lactation was at an even higher level (13–15 gm. per rat per day). The increased amount of food eaten corresponds to that consumed by the control mothers under similar circumstances. At least 10 gm. of the calcium-supplied synthetic diet per rat per day was eaten by the control rats under ordinary conditions. Calcium-deficient weaned young consumed only 2–4 gm. of the deficient ration per rat per day.

5. Chemical composition

Hemoglobin. After giving birth to young the hemoglobin content of the blood of both calcium-deficient and control mothers was below normal. Calcium deprivation had a more pronounced effect on the hemoglobin, however. The amount of hemoglobin of both the deficient and control mothers decreased during the lactation period and was restored to normal about 4 weeks after weaning. Calcium-deficient and control young presented the same blood hemoglobin picture. As the rats grew older, the hemoglobin gradually rose from a low value at birth to the customary level reached at 6–7 weeks of age. Table 1 shows the changes in hemoglobin content of the blood as the result of pregnancy and lactation.

TABLE 1

Hemoglobin concentration of the blood [1]

| | MOTHERS | | YOUNG | |
	Calcium low	Control	Calcium low	Control
Before pregnancy	12.7 (13) [2]	13.5 (3)		
At birth	9.8 (12)	11.3 (7)	9.3 (4)	8.5 (6)
At weaning	8.8 (2)	10 6 (2)	11.3 (6)	11.3 (5)
Four weeks after weaning	12.7 (2)	13.2 (2)	13.0 (2)	13.5 (2)

[1] The data obtained by the Newcomer method ('23) represent average values expressed as grams hemoglobin per 100 ml. blood. The length of time the mothers were maintained upon the calcium deficient diet varied from 26 to 247 days.

[2] The number of cases from which the mean is calculated.

Serum calcium. The serum calcium concentrations are recorded in table 2. The concentration of serum calcium of mature female rats was maintained at the normal level, even though they were deprived of calcium for as long as 275 days. However, if rats were subjected to the additional strain on their calcium stores as the result of pregnancy, the serum calcium concentration was reduced, but never to the low value observed for young growing rats. During the lactation period there was a further slight decrease in the serum calcium concentration of the deficient mothers. The level of the serum calcium of the control mothers never fell below the normal

range. The average serum phosphorus concentration of calcium-deficient mothers was 3.9 mg. per 100 ml. Bodansky and Duff ('41) report similar values for mothers maintained on a low calcium diet (0.017% Ca). The level of the serum calcium concentration of the deficient mother's young remained essentially normal, although it tended to decrease during lactation and was below the corresponding value for

TABLE 2

Serum calcium concentration.[1]

	CALCIUM LOW		CONTROL	
	Range	Mean	Range	·Mean
Mothers				
Before pregnancy [2]	9.2–11.0 (5) [3]	10.0±0.30 [4]		
Immediately post partum [5]	7.1– 9.4 (5)	8.2±0.40	9.1–10.7 (6)	10.3±0.24
During lactation [6]	6.9– 8.9 (5)	7.9±0.33		
Recovered after pregnancy [7]	9.2– 9.5 (2)	9.4±0.16		
Young [8]	8.9–10.2 (7)	9.6±0.17	10.0–12.3 (8)	10.9±0.29

[1] The data are expressed as mg. Ca per 100 ml. serum. The determinations were made upon individual rats by the method of Greenberg and Miller ('41). Approximately 0.05 ml. serum, obtained from the tail without sacrificing the animal, was used for each analysis. We wish to acknowledge to W. D. Miller our appreciation for carrying out the individual serum calcium analyses.

[2] Mothers, after giving birth to one litter, were transferred from the stock colony to the low calcium diet for a period varying from 153–275 days, during which time they were mated twice without success.

[3] The number in parentheses indicates the number of cases from which the mean was calculated.

[4] Mean deviation of the mean.

[5] Mothers had been on the low calcium diet for a period varying from 80–168 days. In only two instances were the deficient young alive at birth. The control mothers gave birth to living young.

[6] Data from two mothers that nursed three young each. Mothers gave birth to eight and seven rats after 39 and 117 days on the calcium-low diet, respectively. Analyses performed on blood drawn between 2–29 days after birth of young.

[7] Analysis performed 43 and 221 days after mothers gave birth to a litter of seven living and seven non-living young, respectively. The first mother nursed three young for a 4-week lactation period.

[8] Age of the young rats varied from 2–39 days for the deficient, and 2–46 days for the control animals.

the control young. Presumably the mother was able to supply enough calcium in her milk for the serum calcium to be maintained at an almost normal level in the young.

Body calcium. As a result of dietary calcium deprivation, the amount of calcium in the carcasses of mature female rats was reduced to the same extent whether or not they bore young. The decrease in total body calcium was gradual. Pregnancy did not appear to influence markedly the reduction in the calcium percentage, but low values were found following lactation. The length of time the rat was maintained on the calcium-inadequate diet was a more important factor than pregnancy and lactation in the determination of the amount of body calcium. The phosphorus content of the entire carcass of the calcium-deprived rat remained at the normal level. Control mothers showed normal body calcium and phosphorus contents after pregnancy and lactation (table 3).

The body calcium content was lower than normal in the young born of mothers fed the low calcium diet. The percentage of calcium gradually increased until at weaning time (28 days of age) it was almost at the normal level. The amount of body calcium of control young rats continued to increase with the growth of the animal, but that of the deprived young even fell off after 4 weeks of age, so that there was a decided difference in the calcium content of 6- and 7-week old deficient and control rats. The former had only about two-thirds the normal body calcium percentage. These differences are illustrated in table 3.

The total phosphorus content of infant calcium-deficient and control rats increased gradually with the growth of the rats. The low calcium young had a phosphorus percentage slightly lower than that of the controls, but it was nevertheless within the normal limits reported by other workers. There was no significant difference between the total body calcium and phosphorus contents of stock colony young and the infant rats reared on the control synthetic diet.

The fetuses from two calcium-deficient mothers just before term had very low calcium but almost normal phosphorus per-

TABLE 3

Total body calcium and phosphorus [1]

	CALCIUM-DEFICIENT		CONTROL		STOCK COLONY	
	Ca	*P*	*Ca*	*P*	*Ca*	*P*
Mothers		550[2](8)				
After 14 weeks on low calcium diet [3]	816 (6)[4]					
After 28 or more weeks on low calcium diet [3]	596 (10)					
No young while on low calcium diet [6]	691 (8)					
After birth of young [7]	727 (6)					
After lactation [8]	573 (2)		1014 (3)	639 (3)		
Young						
Fetuses	80 (2)	268 (2)				
At birth, living	206 (5)				247 (2)	285 (2)
At birth non-living	129 (5)					
Age in days						
2	199 (2)		336 (4)		273 (2)	325 (2)
7	373 (2)				412 (2)	355 (2)
14	381 (2)	518 (2)	545 (2)		476 (2)	423 (2)
28	773 (3)	535 (3)	703 (2)		820 (2)	627 (2)
42	667 (2)	593 (2)	1065 (2)	675 (2)	977 (2)	627 (2)
56	738 (2)	567 (2)	1121 (2)	647 (2)		

[1] Values are averages expressed in milligrams per 100 gm. fresh rat carcass.

[2] As there was no variation in the total body phosphorus among the categories listed, this figure is the average value for all the calcium-deficient mothers.

[3] This time interval includes a period of pregnancy for each mother and a lactation period for one mother.

[4] The number in parentheses indicates the number of cases from which the mean was calculated.

[5] This group includes a period of pregnancy for two mothers and a period of lactation for one mother. The other rats were mated without success.

[6] These animals were maintained on the low calcium diet for a period varying from 75–247 days, during which time they were mated without success.

[7] These mothers were maintained on the low calcium diet for a period varying from 80–153 days. All gave birth to litters but did not nurse them.

[8] These two rats gave birth to young after 29 and 117 days of calcium deprivation and were sacrificed after 96–287 days of calcium deprivation, respectively.

centages; they would probably not have survived. Apparently only those fetuses which had an almost normal calcium content were carried successfully to term.

This statement is supported by the fact that the calcium-deficient infants that were dead at birth showed a much lower body calcium content than those which survived (table 3). The viable young had only 17% less calcium than the stock infants at birth, whereas the non-living young contained about one-half the normal amount of calcium.

Bone composition. The bones of the control mothers had a normal mineral composition. Furthermore, female rats which were transferred to the calcium deficient diet after having reached maturity had essentially the same percentages of ash, calcium, magnesium and phosphorus in their bones as did the control animals. However, rats which had been maintained on the low calcium diet for a long period of time showed a slight reduction in the per cent of ash and of the calcium in the bone and ash; calcium-deprived mothers showed definite decreases in these respects only if they lactated their young. Pregnancy alone only produced a small decrease in the ash content of the bones. The analytical values obtained for the adult rat bones are recorded in table 4.

According to Bodansky and Duff ('41) demineralization of the bone occurs following pregnancy and lactation, especially if the diet is inadequate in minerals. That even a normally fed rat may draw upon the calcium and phosphorus reserves of the trabeculae of the long bones in response to lactation is seen from the observations of Goss and Schmidt ('30) that almost invariably a negative calcium balance occurs during lactation.

The analytical data on the composition of the bones of the young from calcium-deficient and control mothers are recorded in table 5. The composition of the bone ash of the control young showed the usual calcium, magnesium and phosphorus relationships. However, the per cent of bone ash, and, therefore, the percentages of calcium, magnesium and phosphorus in the bones of the young were low at birth and only tended

TABLE 4

Composition of bone of adult rats.[1]

ANIMALS	ASH	CALCIUM		MAGNESIUM		PHOSPHORUS	
	% of bone	% of ash	% of bone	% of ash	% of bone	% of ash	% of bone
Calcium-deficient mature females— no young (7)[2] (75–247 days)[3]	Range 62.2 – 69.0	32.4 – 39.3	21.3 – 25.8	0.49 – 0.82	0.34 – 0.51	13.7 – 18.0	8.7 – 11.7
	Mean 65.8± 1.1	36.5±0.87	24.0±0.66	0.69±0.048	0.46±0.03	15.9±0.59	10.4±0.42
Calcium-deficient mothers — after birth of young (4) (80–153 days)	Range 58.0 – 66.5	35.8 – 39.6	22.7 – 29.1	0.68 – 0.98	0.43 – 0.65	15.3 – 16.4	8.0 – 10.4
	Mean 62.3± 2.0	38.0±0.80	25.0±1.5	0.79±0.066	0.49±0.053	15.8±0.23	9.8±0.35
Calcium-deficient mothers — after lactation (3) (96–146 days)	Range 48.2 – 56.2	31.8 – 36.7	15.9 – 20.6	0.55 – 0.97	0.31 – 0.49	15.8 – 18.4	7.6 – 10.3
	Mean 51.6± 2.4	33.8±1.5	17.3±1.6	0.75±0.12	0.39±0.054	17.5±0.85	9.0±0.78
Control mothers — after lactation (3) (111–165 days)	Range 61.7 – 72.6	35.5 – 38.7	21.9 – 28.1	0.68 – 0.75	0.44 – 0.54	16.3 – 17.0	10.6 – 12.3
	Mean 66.0± 3.3	37.2±0.93	24.6±1.8	0.73±0.024	0.49±0.029	16.7±0.22	11.9±0.52

[1] The bone analyses were made upon the two femurs of the rat. Data are expressed as per cent of dried, defatted bone and of ash. The number in parentheses indicates the number of cases from which mean was calculated. Measure of variability is mean deviation of the mean.

[2] The number in parentheses indicates the number of cases from which mean was calculated. Measure of variability is mean deviation of the mean.

[3] The time on the experimental diet expressed in days.

TABLE 5

Composition of bone of young rats [1]

AGE IN DAYS	ASH	CALCIUM		MAGNESIUM		PHOSPHORUS	
	% of bone	% of ash	% of bone	% of ash	% of bone	% of ash	% of bone
Calcium-deficient young							
0	17.5	49.8	8.72	1.29	0.23	18.1	3.2
10	20.2	35.2	7.10	0.89	0.18	12.5	2.5
16	35.4	24.6	8.69	1.20	0.43	16.0	5.6
22	25.0 [2]	33.6 [2]	8.40	0.95 [2]	0.24	20.3 [2]	5.1
29	25.0 [2]	33.8 [2]	8.45	1.13 [2]	0.28	17.3 [2]	4.3
29	34.0	40.2	13.80	0.79	0.27	15.0	5.2
39	24.0	34.3	8.23	0.83	0.20	21.5	5.2
45	25.0	32.2	8.04	0.94	0.24	20.5	5.1
57	18.9	45.6	8.62	1.17	0.22	17.6	3.3
Control young							
11	57.2	33.0	18.9	0.60	0.34	15.8	9.0
11	56.9	39.6	22.5	0.71	0.40	15.5	8.8
28	41.5	38.1	15.8	0.79	0.33	18.8	7.3
32	46.2	39.6	18.3	0.72	0.33	17.2	8.0
46	55.3	43.2	23.9	0.74	0.41	18.3	10.1
47	61.7	37.0	22.8	...	...	17.3	10.6
53	64.5	41.9	26.6	0.67	0.43	15.6	10.1

[1] The bone analyses were made upon the two femurs of the rat. Data are expressed as per cent of dried, defatted bone and of ash.

[2] Very low ash percentages were obtained (10.8 and 9.3%, respectively) for these analyses. As these appeared to be in obvious error, due to loss of ash during the preparation of the bones for analysis, the average value for the per cent of ash of the remaining results was substituted for these two figures in the calculation of the amount of calcium, magnesium and phosphorus in the ash.

to approach the adult level with the growth of the rats. The results are comparable to those obtained by Orent, Kruse and McCollum ('34) for the composition of normal bone as influenced by the growth of the rat.

The bone analyses of the low calcium young presented an interesting picture. The percentage of ash was extremely low at birth. It subsequently showed a slight rise while the rats were being fed by the mother, and then dropped off to the previous level after the rats were weaned. The bones of these young rats were practically indistinguishable from the soft tissues of the body. They were almost transparent and simply

shells for the bone marrow. They were not even comparable in consistency to normal cartilage, but were much less firm and thinner. Normal rat bones at birth, of course, are mainly cartilaginous, and most of the calcification as well as the ossification occurs during the growth of the rat. Because of the minute size of the young bones and their extreme fragility of texture, it was not easy to prevent loss of ash during the preparation of the bones.

The percentages of calcium and phosphorus in the bone ash were about normal, and the percentage of magnesium somewhat higher than normal. This agrees with the observation of Hammett ('23) that the calcium, magnesium and phosphorus make-up of bone ash is always approximately the same regardless of the amount of ash present in the bone. There is a strong tendency for the organism to maintain a balance between the various mineral elements, so that the ash is almost uniform in percentage composition. Bodansky and Duff ('41) also have noted that the bone ash composition is constant under variable conditions of diet. These findings are favorable to the hypothesis that the mineral matter of bone is made up, in the main, of a single, complex compound.

On the other hand, the calcium percentage of the bone at birth was only about one-third the normal adult value. This same percentage of calcium was maintained by the animals throughout their growth. No more calcium was added to the bones of the calcium-deprived young during the lactation period or during the first few weeks after weaning. After the young rats were deprived of their source of calcium from the mothers' milk, they had to use their own meagre stores of calcium in order to meet life's needs. Hence, the percentage of bone ash was markedly reduced after weaning. The amounts of magnesium and phosphorus in the bones were also much lower than the corresponding control values. The whole general bone picture of the low calcium young was similar to that observed for growing rats transferred to the calcium-inadequate diet after weaning. The differences between the control and deficient bones were greatly accentuated, however,

in the case of the young rats from mothers also deprived of calcium.

Cox and Imboden ('36) note that female rats, even though maintained on experimental diets of widely variable mineral content, nevertheless give birth to young having constant mineral composition. Their conclusion is that a change in dietary minerals is not immediately reflected by change in the composition of the fetal tissue, and, conversely, that the maternal organism is able to regulate the mineral elements supplied to the fetus. Furthermore, gestation per se is little strain on the rat mother as compared to the human mother, because rat young are cartilaginous at birth. However, a 21-day old rat approximates the human infant at birth with respect to its calcium skeletal content, so that lactation is definitely an added strain upon the rat mother. On the other hand, Bodansky and Duff ('41) point out that the average litter weight at birth is one-fifth to one-fourth the weight of the mother rat, so that the total calcium of a normal litter is about 5% of the maternal total calcium, whereas the total calcium of a human infant is only approximately 1.5–2.5% of the maternal calcium.

The observations made in this study indicate that lactation is decidely a greater strain than is pregnancy upon the calcium stores of the female rat. However, it should be pointed out that in only a small per cent of the cases of the present investigation was the deficient mother able to provide the normal requirement of calcium for the fetus and, hence, give birth to normal living young. Furthermore, the drain on the calcium stores of the mother during lactation was reduced, not only by the lack of deposition of calcium in the bones of the baby rats, but also by the restriction of the size of the young rats. The maternal organism attempted to produce normal young insofar as that was possible and still maintain its own essential calcium supply.

SUMMARY

1. Rats reared from weaning time on a synthetic diet containing only about 10 mg. calcium per 100 gm. food failed to mate.

2. After being transferred to the diet low in calcium, fertility was markedly decreased among a group of mothers which had borne a previous litter each. The number of viable young produced was very low.

3. The mothers and young were subject to the consequences of extreme calcium deficiency as evidenced by their susceptibility to hemorrhages, prostration and paralysis induced by a galvanic stimulus. The symptoms were greatly exaggerated in severity for the young.

4. Pregnancy was not a great drain upon the calcium stores of the mother rat, but lactation definitely reduced the amount of skeletal calcium. This was shown by a lowering of total body calcium content, serum calcium concentration, percentage bone ash and per cent of calcium of bone and ash. These changes were magnified by a marked loss in weight by the mother during lactation as contrasted to a maintenance of body weight during gestation.

5. Calcium-deficient young that were able to survive, were almost normal at birth except for a low bone ash and bone calcium content. The deficient mother was able to supply some calcium to the young during the lactation period but not enough to maintain the normal calcium content of the skeletal structures. She was not capable of providing enough milk to allow for the normal growth of her young.

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DIGESTION OF WHOLE WHEAT AND WHITE BREADS IN THE HUMAN STOMACH *

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The experiments of Russell and Nasset ('41) and of Bly, Heggeness and Nasset ('43) from this laboratory have demonstrated that both motility and secretory activity of the upper gastrointestinal tract in dogs are plainly affected by certain of the B-complex factors, especially pantothenic acid. Animals deficient in this factor required a longer time to evacuate a meal and to digest it to the same degree than did animals receiving whole yeast. It occurred to the writers that one reason why many people do not learn to appreciate whole wheat bread might be a relative paucity or unavailability of pantothenic acid in such breads as compared with white breads. If this factor were essential to gastric motility or to the normal rate of secretion, if indeed it should prove to be an important secretogogue, for man also, slow digestion resulting from its lack might contribute to the palling of appetite for a food with this deficiency.

One way of testing such an hypothesis would be to compare in human subjects the rates of gastric digestion of a whole wheat bread eaten both with and without the addition of Ca-pantothenate. The latter rates might also be compared with those for the same foodstuffs in the same subjects eating equivalent amounts of white breads.

It would obviously be important in testing the rate of digestion of starch that the same length of time be consumed in chewing the several breads, and, for comparison of any speci-

fied digestion, that reasonably equal amounts of the gastric contents be recovered at the same interval after eating.

Because the laboratory had already gained considerable knowledge of the "peeled-wheat" bread (Murlin, Marshall and Kochakian, '41; Sealock, Basinski and Murlin, '41), it was chosen to represent the truly whole wheat breads in this study, for the flour from which it is baked has had nothing removed except the outermost epidermis of the whole grain, and no other source of starch or protein is added. For comparison, the same bread baked with high-vitamin [1] yeast, and two white breads — one made of 76% extraction flour plus 3½% dry milk solids and baked with high-vitamin yeast, and one made of lower [2] extraction flour containing a somewhat lower percentage of dry milk solids and baked with ordinary yeast — were chosen.

EXPERIMENTAL

Six reliable graduate students were selected as subjects. It was planned to give test meals of peeled-wheat bread to each of these subjects over a period of days until reasonably constant results were obtained as regards the soluble N and free sugar found in samples taken at 1-hour intervals from the time of eating. At the start, the Rehfuss tube was swallowed before the test meal was eaten so that freedom from solids remaining in the stomach could be confirmed. The tube was then left in place while the test meal of bread was taken and for 1 hour longer. However, it was necessary to abandon this procedure for four reasons aside from the difficulty of accomplishing a thorough mastication with the tube in place: (1) Three of the subjects began to develop low gastric acidity within the first week and two of them reached the point of having no free acid in the second week; (2) the "bucket" of the tube became completely clogged so that a freely flowing sample could not be drawn without using excessive quantities of water, which was obviously undersirable; (3) the x-ray pictures taken after barium-mixed meals on days alternating

[1] See p. 133 for latest analysis of this yeast.
[2] About 72% of the entire wheat berry, according to the manufacturer.

with the digestion-test days showed some irregularties of motility attributable, it was thought, to variation in lodging place of the "bucket"; (4) two of the subjects experienced considerable pain and traces of blood were found in their gastric contents. These two persons therefore were excused. Two fresh subjects were selected and a new start was made, after the lapse of a week, to afford a complete rest for the four veteran subjects who had experienced no symptoms.

Under the new regime each subject was instructed to drink a glass of water immediately on arising on the test day and to report at the laboratory in time to begin eating the test bread approximately 1 hour after taking the water. This schedule was observed regularly throughout the remainder of the study. To avoid possible injury to the gastric mucosa from too frequent use of the stomach tube, the tests from June 15 on were limited to 4 successive days the first week and 3 each week thereafter. The subjects started to eat the new bread for each week on Monday at the rate of two slices each meal. The test meal consisting of 150 gm. (five to six slices) fresh weight of each bread, together with 250 ml. of unsweetened clear tea, was given Wednesday [3], Thursday and Friday mornings. At the other two meals on these days the regular two slices were eaten. On Saturdays and Sundays the special breads were omitted and laboratory work was not done. This schedule was tolerated perfectly.

The actual eating of the test meal each time occupied just 30 minutes, with one single exception. This provided none too much time for thorough insalivation but was adhered to for the sake of regularity.

In the second period each subject was given two measured doses of 16 mg. each of Ca-pantothenate with instruction to take one of them on retiring the night before the test meal was to be taken and another 1 hour before the time fixed for the test meal, early in the morning (see footnote 6).

As nearly as possible, 1 hour after completion of the meal the sample of grastric contents was drawn through the Rehfuss

[3] Except for the first period, June 16 to 19, when the test meal began on Tuesday.

tube which had been swallowed immediately before. Variations from the 60 minutes' standard interval were occasioned by plugging of the tube. Corrections were made for these variations as well as for the preformed soluble N and free reducing substances in the breads for the purpose of statistical analysis of the results (see below).

METHODS

After the removal of aliquots for pepsin determination by the Mett method, all samples were immediately boiled to destroy enzyme action, then homogenized, diluted to precise volumes and partitioned for determination of total solids, total acidity, free acidity, total reducing substance after hydrolysis with HCl, free reducing substance, total nitrogen, and soluble nitrogen, by well-known standard procedures.

RESULTS

In table 1 are shown the results of the gastric analyses averaged for the 3 (or 4) days in each period for each subject. The values for any given substance shown by any single individual were surprisingly consistent from period to period. For example, the amount of total solids in the sample was always highest in the gastric contents from subject 6. This was due to a large percentage of mucus. The highest total acidity was shown in every period by subject 2, with subject 1 always a close second. These two subjects also showed the highest free acidity, while the lowest value on peeled-wheat breads appeared in the sample for subject 6. Subject 2 exhibits in every period the lowest average free sugar, a circumstance doubtless related to his high free acidity. It is reasonable to suppose that the low pH in the gastric contents retarded starch digestion by ptyalin. Correspondingly the highest values for free sugar in the gastric contents accompany low values for free acidity. High values for pepsin would be expected to accompany high free acidity and such proved to be the case. Further, low values were associated with low acidity and medium values with medium acidity.

TABLE 1
Analysis of stomach contents.

SUBJ.	VOL. OF SAMPLE	TOTAL SOLIDS	TOTAL ACIDITY	FREE ACID	REDUCING SUBSTANCE		NITROGEN		PEPSIN: METT TUBE VALUE
					Total	Free	Total	Soluble	
	ml.	*gm./100 ml.*	*ml./N/10 per 100 ml.*	*ml./N/10 per 100 ml.*	*mg./gm. dry solids*		*solids mg./gm.*		
Period 1 — June 16–19 (Peeled wheat bread)									
1	247	7.90	95.8	83.4	559	147.3	27.02	12.89	19.3
2	226	8.49	103.0	90.0	557	108.4	26.68	13.72	20.2
3	192	6.77	68.9	57.6	538	152.7	26.88	12.73	14.1
4	277	7.36	88.4	76.0	529	132.4	26.94	13.36	11.9
5	235	7.80	80.7	68.2	568	151.7	25.18	11.48	11.9
6	221	12.48	80.4	56.3	584	131.0	27.26	12.56	10.7
Av.	233	8.46	86.2	71.9	556	137.3	26.66	12.79	14.7
Period 2 — June 24–26 (Peeled wheat bread after ingestion of Ca pantothenate)									
1	259	6.49	95.3	86.3	493	135.3	26.10	14.07	19.2
2	245	9.07	99.7	86.0	566	106.1	25.44	14.03	20.8
3	169	7.51	81.9	70.0	561	145.6	26.10	12.45	17.2
4	276	7.41	79.4	63.0	576	144.1	27.46	14.60	15.4
5	252	6.34	68.4	58.7	560	166.1	25.74	13.42	10.7
6	233	12.05	71.8	49.1	581	144.4	28.07	14.72	9.7
Av.	239	8.14	82.1	68.8	556	140.3	26.48	13.88	15.5
Period 3 — July 1–3 (Peeled wheat bread with high-vitamin yeast)									
1	270	5.77	99.1	87.8	518	133.4	28.02	14.43	18.1
2	173	7.43	101.5	87.8	533	120.0	30.24	16.37	22.8
3	197	8.03	86.3	69.7	549	154.1	26.73	12.55	16.4
4	279	6.92	77.9	65.8	563	148.8	27.06	14.69	11.3
5	207	8.04	80.2	64.8	567	175.4	27.40	12.50	14.2
6	220	13.83	80.1	55.9	542	138.4	27.30	13.52	10.8
Av.	224	8.33	87.5	71.9	542	145.0	27.79	14.21	15.9
Period 4 — July 8–10 (High-extraction white bread)									
1	267	5.83	96.2	86.7	604	124.0	30.6	14.99	15.1
2	240	9.88	99.2	86.3	616	90.7	24.7	15.97	15.6
3	139	7.04	60.6	51.4	624	135.1	25.7	13.40	11.1
4	237	11.97	64.2	53.9	617	130.9	24.1	14.85	7.5
5	165	12.13	67.6	55.8	602	162.0	23.5	12.24	9.1
6	226	17.75	70.7	57.6	628	121.7	23.7	15.45	7.8
Av.	212	10.76	76.6	65.3	615	127.4	25.4	14.48	11.0
Period 5 — July 15–17 (Ordinary white bread)									
1	203	7.19	88.4	79.4	646	127.4	25.1	18.4	15.8
2	236	13.22	89.0	78.5	623	94.2	23.6	16.2	14.5
3	144	10.37	64.9	54.2 [1]	650	145.6	23.6	13.8	11.4
4	163	13.30	63.5	50.1	658	148.7	24.5	15.1	8.4
5	117	14.23	65.6	49.9	612	163.2	22.7	15.9	9.5
6	211	18.74	70.3	53.2	673	121.0	23.2	14.4	7.7
Av.	179	12.95	73.6	61.3	643	137.0	23.3	15.7	11.2

[1] Average of only 2 days.

When the daily results were examined for the first three periods comprising sixty individual test meals — forty-two on peeled-wheat bread, and eighteen on the same baked with high-vitamin yeast and differing only slightly in composition from the former — the values showed a perfectly random distribution not affected by any systematic error. This fact bears out the consistency of the results for each subject in the average analytical values of table 1. The close agreement for total solids per 100 ml. of stomach contents among the three average values in these periods, and among the average values for free acidity and pepsin in the stomach contents, is evidence not only that the subjects were performing consistently, but also that the error of determination was satisfactorily low and uniform. The agreement, also, in all these average values between the results from the two white breads is as good as could be expected in view of their differences in composition (table 2).

TABLE 2

Analysis of breads for reducing substances and nitrogen.

BREADS	REDUCING SUBSTANCE		NITROGEN	
	Total	Free	Total	Soluble
	mgm. per gm. dry wt.		*mgm. per gm. dry wt.*	
Peeled whole wheat bread	638	71.5	26.81	2.52
Peeled whole wheat bread (Period 2)	622	69.5	27.07	2.73
Peeled whole wheat bread (baked with high-vitamin yeast)	637	70.7	27.07	2.76
High extraction white bread	685	43.8	24.32	2.36
Ordinary white bread	667	52.4	24.40	2.20

The values which reflect the speed of digestion, however, are contained under the headings "Reducing Substance" and "Nitrogen", and consistency among them is the resultant of composition of the food material as this may influence digestibility, as well as performance of the gastric glands. Hence, we do not find the figures running parallel in different columns so closely as they do under free acidity and pepsin (table 1). Nevertheless, it is perfectly evident that high acidity and

high pepsin in the first two periods on the same bread were associated with more soluble nitrogen than were low acidity and low pepsin; and conversely, that high acidity tended to retard, and low acidity to favor, free sugar (reducing substance) formation from starch.

These values, however, are subject to two corrections: (1) variations in the time of sampling, and (2) the occurrence of soluble nitrogen and free reducing substance in the breads as eaten. The variations in time have already been explained. In some periods they were large enough to affect considerably the amount of digestion from the time the food was eaten until the drawn sample was boiled to stop the enzyme action. Whether the taking of 16 mg.[4] of calcium pantothenate several times before eating the peeled-wheat bread (see below) produced any significant change in rate of digestion as compared with the same bread eaten alone could not be established certainly without making these corrections.

For example, the difference between the average values for soluble nitrogen in periods 1 and 2 (table 1) is 1.09 mg. per gram of solids in the stomach contents, or about 8.5%. Applying the statistical analysis of Fisher ('36) for the difference between two means to the two sets of individual determinations — eighteen for each period — the probability (p) that this difference is due to chance, is less than .01, i.e., less than 1 in 100. It must therefore be due to the fact that calcium pantothenate increased the speed of digestion; there is no other factor to which it could be ascribed. However, this analysis is based on the uncorrected figures. Would the difference be increased by using corrected values?

In table 3 are shown the effects of making the two corrections on the original values for 3 days of period 2. In arriving at the corrections for time, the average values obtained for elapsed times (from 60 to 70 minutes) with the peeled-wheat bread alone between completion of the meal and completion of sample were plotted and were found to fall in a straight line.

[4] This dose was chosen because an equivalent amount per kilogram of body weight had proven effective in the deficient dogs (Bly, Heggeness and Nasset, '43).

TABLE 3

Sample corrections of individual values for time and preformed substances in bread [1] (all values in mg./gm. solids).

	SOLUBLE N				FREE SUGAR		
Orig-inal crude value		Corrected		Orig-inal crude value		Corrected	
	For time	For sol. N in bread	Correct value		For time	For free sugar in bread	Correct value
16.14	...	2.73	13.41	174.7	..	69.5	105.2
14.85	—0.56	2.73	11.56	147.8	—3.2	69.5	75.1
11.22	...	2.73	8.49	152.2	—3.2	69.5	79.5
15.46	...	2.73	12.73	171.7	—6.4	69.5	95.8
12.86	+0.28	2.73	10.41	142.4	+3.2	69.5	76.1
13.87	—0.84	2.73	10.25	109.8	+3.2	69.5	43.5
13.45	—0.28	2.73	10.44	172.1	..	69.5	102.6
11.72	+0.28	2.73	8.71	141.1	..	69.5	71.6
12.19	—0.28	2.73	9.19	155.5	—3.2	69.5	82.8

[1] The days corrected for are chosen at random to show the range of original values and of corrections for time.

From this graph the corrections per minute were derived. With one of the white breads the corrections for time were not possible for two reasons: (1) on account of the higher relative content of gluten the stomach contents were inclined to form clumps, thus obstructing the sampling tube more and thereby producing greater variations in the sampling time which were not due to mere delay in getting started; and (2) there were some obvious errors in recording the times for this period. It turns out, however, that the difference between the mean value for this period and the other white bread period, as well as between periods 5 and 1 (peeled-wheat bread eaten alone) was great enough to be just as significant without this correction as with it (judging by the effect of such correction in period 4).

Time corrections, it will be noted (table 3), are sometimes positive but more often negative, meaning that the time of drawing the sample was longer than 60 minutes from the time the meal was finished more times than it was less than 60. The corrections for the soluble N and free sugar were based on the average of several analyses and are constant. The re-

sult is that the difference between the two means discussed is increased from 1.09 mg. to 1.33 mg. per gram sample.

Applying statistics to all the individual corrected values the results are as shown in table 4. The percentage difference between the two means for soluble N in periods 1 and 2 is now 14.9% instead of 8.5% but the probability value (p) is not changed (Fisher's table stops at .01).

TABLE 4

Summary of digestion data for the several breads and signicance of difference between means.

PERIODS COMPARED	BREADS COMPARED	SOLUBLE N				FREE REDUCING SUBSTANCE			
		Corrected means compared		% difference	p	Corrected means compared		% difference	p
		mg./gm. solids				*mg./gm. solids*			
1 and 2	P.W.[1] bread and same + Ca pant.	8.91	10.24	14.9	.01	64.6	71.1	10.0	.01
1 and 3	P.W.[1] bread and same with hi-vitamin yeast	8.91	10.28	15.3	.01	64.6	74.1	14.7	.01
1 and 4	P.W.[1] bread and high-extraction white bread with hi-vitamin yeast	8.91	10.93	22.5	.01	64.6	78.3	21.2	.01
1 and 5	P.W.[1] bread and ordinary white bread	8.91	13.10	45.9	.01	64.6	79.3	22.7	.01
4 and 5	High-extraction and ordinary white breads	10.93	13.10	19.8	.01	78.3	79.3	1.3	not sig.

[1] P.W. for "peeled wheat".

The comparisons between amounts of digestion with peeled-wheat bread and the same eaten with calcium pantothenate, on the one hand, and peeled-wheat bread and the same baked with high-vitamin yeast on the other, in periods 2 and 3, show practically no difference (10.24 and 10.28 mg. per gram solids (table 4)). This means the advantage gained by eating peeled-wheat bread baked with high-vitamin yeast is matched by eat-

ing the same bread baked with ordinary yeast after taking
calcium pantothenate twice before the meal.

	FOIL YEAST (DRY BASIS) $\mu g./gm.$	HIGH-VITAMIN YEAST (DRY BASIS) $\mu g./gm.$
Thiamine	128	700
Riboflavin	40	65
Nicotinic acid	300	340
Pyridoxine	20	40
Pantothenic acid	94	100 to 150 (mean 125)

Vitamin values for the yeasts [5] are given here based on the
latest analyses. They do not necessarily represent the true
values for the ordinary bakers' yeast and the high-vitamin
yeast used in baking the two varieties of peeled-wheat bread
tested in this investigation, because different batches of both
yeasts have shown appreciable variations. They do serve,
however, to give an approximate idea of the amount of extra
vitamins obtainable from eating 150 gm. of the high-vitamin
yeast bread as compared with the peeled-wheat bread baked
with ordinary yeast. It is not likely that single selected
batches of the two yeasts would present greater differences.
From the figures for pantothenic acid given above, therefore,
it may be calculated that the person eating 150 mg. of high-
vitamin peeled-wheat bread would obtain 0.45 mg. more of this
vitamin than he would when eating the same bread baked with
ordinary yeast.

From determinations made in this laboratory (Sealock and
Livermore, '43) the peeled-wheat bread baked with ordinary
yeast contains 5.2 mg. of pantothenic acid per gram or 0.78 mg.
in 150 gm. If we may assume that pantothenic acid is the only
one of the B-factors affecting rates of digestion (Russell and
Nasset, '41), then it appears that the addition of less than 1
mg. of this vitamin in the natural form in the bread, produces
effects on digestion of protein and carbohydrates equal to or
greater than the effects of over 15 times as much of the syn-
thetic calcium salt taken as an ''appetizer'' an hour before
the meal (first two comparisons in table 5).

TABLE 5

Comparison of digestibilities of several breads. Soluble N and free sugar formed in 1 hour as percentages of insoluble N and total hydrolyzable carbohydrate in breads.

BREADS COMPARED	PERIODS	SOLUBLE N'S AS % OF TOTAL INSOLUBLE N IN BREAD		DIFFER- ENCE IN RATE %	FREE SUGAR AS % OF TOTAL HYDROLYZABLE CARBOHYDRATE		DIFFER- ENCE IN RATE %
Peeled wheat bread and same eaten after Ca pantothenate	1 & 2	36.6	42.1	15.0	11.8	12.9	9.3
Peeled wheat bread and same baked with high-vitamin yeast	1 & 3	36.6	42.3	15.6	11.8	13.1	11.0
Peeled wheat and high ex- traction white bread	1 & 4	36.6	49.8	36.1	11.8	12.2	3.4
Peeled wheat bread baked with high-vitamin yeast and high extraction white bread	3 & 4	42.3	49.8	17.7	13.1	12.2	— 6.9 [1]
Peeled wheat bread and ordinary white bread	1 & 5	36.6	59.0 [2]	61.2	11.8	13.1	11.0
Peeled wheat bread baked with high-vitamin yeast and ordinary white bread	3 & 5	42.3	59.0	39.5	13.1	13.1	0.0
High extraction white bread and ordinary white bread	4 & 5	49.8	59.0	18.5	12.2	13.1	7.4

[1] With this single exception the rate of digestion of the second member of each pair is higher.

[2] The more rapid digestion of protein is due to higher relative gluten content in the ordinary white bread, and to the dry milk solids.

This is a surprising and gratifying result, but one must not conclude too hastily that no other factor in the high-vitamin yeast has contributed a share to the more favorable effect. There is, for example, in the work of Bly, Heggeness and Nasset ('43) some evidence that small amounts of inositol produce in dogs a measurable acceleration (+14%) in the digestion rate of the carbohydrate in peeled-wheat bread. Another view, which equally commends itself, is that when a small addition of a vitamin proves adequate to produce a full digestion rate any addition above this adequate level is ineffective. It is clearly possible that 1 mg. represents the adequate dose for pantothenic acid.

The other comparisons in table 4 will be self-explanatory. It is of interest that, with respect to the amount of soluble N formed in 1 hour, the high extraction white bread baked with high-vitamin yeast excels the peeled-wheat bread more than does the peeled-wheat bread baked with this yeast. Probably the finer texture of the white bread and the higher relative gluten content make the différence. The standard or "ordinary" white bread is highest in the percentage of soluble N formed. The percentage difference between it and the peeled-wheat bread is twice that between the high extraction white bread and the peeled-wheat bread (table 4). This probably is due to the slower rate of digestion of proteins contained in the aleurone cells and other more fibrous portions of the grain, in the high extraction bread.

None of the comparisons on the formation of free sugar from values corrected in the same manner as those for soluble N reveals differences as large as the corresponding values for digestion of protein. In general, however, they fall in the same order. Only the difference between high extraction white bread and the ordinary white bread is not significant.

In preparation of the necessary values for comparison of rates of digestion, the amounts of insoluble N (protein) and total hydrolyzable carbohydrate present in the several breads when they were eaten have been taken into account. These quantities are derived from the data in table 2, and when the corrected values for soluble N and free sugar (table 4) are expressed as percentages of such quantities, we have the relative rates as between the several pairs of breads (table 5).

For the same comparisons (table 4) the differences between the rates per 100 parts of protein per hour (table 5) are always of the same order of magnitude as the corresponding differences in absolute amounts per hour (table 4). This might mean, of course, that the perecentage of protein in the several breads was equal, but from table 2 it is evident that the whole wheat bread contains nearly 3% more protein [6] than the two

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[6] It is well-known that a small portion of the insoluble N in whole wheat does not represent true protein. (See Winton and Winton, Structure and Composition of Foods, Vol. I, Cereals, 1932, p. 211, et seq.).

white breads which happen to be nearly equal, and some of it is less readily available for digestion. Not knowing what this correction is, we must conclude from the figures as they stand that a larger percentage of the protein in the white breads than in the whole wheat breads is rendered soluble in the same length of time by gastric digestion.

This is not true of the amylolytic digestion of starch by salivary amylase in the stomach. For example, the rate of digestion of high-vitamin peeled-wheat bread by this enzyme is 11% more rapid than that of peeled-wheat bread with ordinary yeast, and the ordinary white breads exceed peeled-wheat bread by exactly the same percentage difference. It follows, of course, that the high-vitamin peeled-wheat breads and ordinary white breads are equal as shown. It is interesting that the whole wheat bread baked with high-vitamin yeast exceeds by a small percentage the high extraction white bread baked with a smaller amount of the high-vitamin yeast.

DISCUSSION

The most significant result among those summarized in table 5 is that a small extra amount of pantothenic acid (possibly aided by some other B-factor) contained in high-vitamin yeast has a favorable effect on digestion at least as great as much larger amounts of the synthetic calcium salt of this acid taken in at least two doses: one, an hour before the test meal, and the other 8 to 10 hours before.[7]

Next in importance is the finding that, with respect to the digestion of starch contained in breads, the use of high-vitamin yeast seems to bring the digestibility of whole wheat bread up to that of ordinary, low extraction white bread. This appears to offer a clue to the preparation of a "successful" whole

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[7] It should be explained that the calcium pantothenate was administered in this manner to avoid adsorption of the compound on barium carbonate which was being used on adjacent days to render visible by x-ray examination any effects the various B-factors might have on gastric motility. There appeared to be good evidence for nullification of such effects by barium compounds. These experiments have been discontinued pending the development of a more suitable opaque medium.

wheat bread, but the improvement as yet applies only to digestion of "hydrolyzable carbohydrate". Possibly the addition of non-fat milk solids would increase protein digestion (in the stomach) also to the level of that of ordinary white bread. Further experiments will be necessary to clarify this and some other questions involved in the production of a wholly acceptable whole (98%) wheat bread.

It is of special interest that in the gastric digests of the whole wheat bread the average pepsin content was approximately 40% greater (Mett tube measurement) than in those of the two white breads. The peptogenic value of whole wheat bread, therefore, is not to blame for lower rates of protein digestion, and such error as results from the small amount of (soluble) nitrogen in the pepsin does not invalidate but rather gives emphasis to the slower rate of gastric digestion of the whole wheat product. The vital economic value of whole wheat as food for man is not touched by these facts, for the over-all digestion is sufficiently high to produce a large net saving not only of protein but also of calories from the wheat for human consumption (Murlin, Marshall and Kochakian, '41).

From this study it appears plausible that the slower rate of digestion of whole wheat in the human stomach as compared with white breads is responsible at least in part for the relative unacceptability of the former by the general public.

SUMMARY AND CONCLUSIONS

Peeled-wheat bread, which is made from flour containing all of the wheat kernel except the outer epidermis weighing less than 2%, has been studied in comparison with several other breads in experiments on gastric digestion in six human subjects. Samples drawn from the stomach by means of the Rehfuss tube 1 hour after eating were analyzed for total and free acidity, total solids, pepsin, total and free reducing substance, and total and soluble nitrogen. Corrections for time lost in sampling and for free reducing substance and soluble nitrogen in the breads made possible the calculation of rates of carbohydrate and protein gastric digestion.

The peeled-wheat bread baked with high-vitamin yeast undergoes gastric proteolytic digestion 15% faster than when the bread is baked with ordinary bakers' yeast; the free sugar formation under the amylolytic action of saliva is 11% faster. Calcium pantothenate in 16 mg. doses taken at least twice before the meal (8 to 10 hours and 1 hour) accelerates the two digestion rates to about the same extent. Pantothenic acid in the test meal of high-vitamin yeast bread exceeded that of the same bread baked with ordinary yeast by only 0.45 mg. If this is the only vitamin B-factor affecting digestion rates, it appears that a relatively small amount in the bread is as effective as a much larger amount taken before the meal.

A "high extraction" white bread containing 3.5% milk solids, based on the flour, and made with high-vitamin yeast, showed a digestion rate for protein and carbohydrate 36% and 3%, respectively, faster than that for the peeled-wheat bread. A white bread made of "straight grade" flour representing an extraction, according to the manufacturer, of "about 72% of the entire wheat berry" and containing 2.5% non-fat milk solids based on the flour showed digestion rates 61% and 11%, respectively, faster than those for the peeled-wheat bread. This white bread, however, was digested only 39% faster than the peeled-wheat bread baked with high-vitamin yeasts with respect to protein, and not at all faster as regards the carbohydrate.

The shortcoming of whole wheat bread does not lie in a lesser peptogenic effect, for the pepsin content in the gastric digests was 40% higher than in those from the white breads.

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THE EFFECT OF SODIUM CHLORIDE UPON THE DISPOSITION OF INJECTED GLUCOSE IN A STRAIN OF RATS [1]

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ONE FIGURE

That sodium chloride is related in some way to the metabolism of glucose is suggested by a number of reports in the literature. The onset of diabetes seems to be followed by an increased excretion of sodium chloride with a consequent depletion of this electrolyte in the body fluids. Adlersberg and Wachstein ('37) observed that both sodium and chloride levels fall in the blood and tissues following pancreatectomy in the dog. This is said to be due to an increased urinary loss of sodium chloride. Following the withdrawal of insulin from human diabetics a large amount of base is lost in the urine and the quantity combined with ketones is much less than the quantity sacrificed with chloride (Peters, Kydd, Eisenman and Hald, '33). Sodium chloride, given in large daily doses, has been reported to have a favorable effect upon the utiliza-

tion of glucose by human diabetics (McQuarrie, Thompson
and Anderson, '36; MacLean, '35). This interesting observa-
tion, however, could not be confirmed by Strouse, Buel, Kay
and Drury ('41).

A diet rich in sodium chloride, when fed to experimental
animals, enhances the deposition of glycogen in the liver
(Crabtree and Longwell, '36), increases the fall in blood sugar
following insulin administration (Adlersberg and Wachstein,
'37; Lewis and Longwell, '41), and improves the glucose tol-
erance of pancreatectomized dogs (Adlersberg and Wachstein,
'37). The tolerance of the pancreatectomized rat to intraperi-
toneally administered glucose is improved when sodium
chloride is given along with the glucose (Orten and Devlin,
'40).

The present experiments were designed to study the effect
of sodium chloride upon the disposition of intraperitoneally
administered glucose in the rat. During the course of the in-
vestigation it was discovered that a high percentage of rats
of the stock colony had a low tolerance to glucose (Orten and
Devlin, '40).

The low tolerance to glucose of the Yale strain of rats has
been the subject of a series of investigations (Cole and Harned,
'38; Cole, Harned and Keeler, '41). These authors have pre-
sented evidence in support of the view that the phenomenon is
related to an hyperpituitary function in this strain (Harned
and Cole, '39). Furthermore, the adrenals of male rats of the
Yale strain are significantly heavier than adrenals of male
Wistar rats (Cole and Harned, '42). Carbohydrate balance
studies indicate that an impairment in the mechanism of gly-
cogenesis or glycogenolysis is the basis for the low tolerance
to glucose (Orten and Sayers, '42). Connected with this ob-
servation is the fact that adrenal-medullectomy is followed by
a normal tolerance to glucose in these animals (unpublished
observations made in this laboratory).

The action of sodium chloride in improving the glucose tol-
erance of the Yale strain of rats has been investigated in the
present study from the standpoint of its effect upon absorp-

tion, excretion, storage and transformation of the administered glucose. The improvement is shown to be the result of an increased storage of glucose as glycogen in the salt-treated animals.

EXPERIMENTAL

The animals used in this study were healthy, adult female rats of the Connecticut Agricultural Experiment Station ("Yale") and Wistar strains, weighing from 200 to 350 gm. Special care was taken to lessen variation in stored carbohydrate due to diversity in food consumption. Three days prior to glucose administration the stock colony ration,[3] which had been given ad libitum up to this time, was fed to the animals at 10:00 A.M. daily in an amount equal to 10 gm. per 250 gm. of body weight ± 1 gm. for each 50 gm. deviation in body weight. The animals were fasted 16 to 18 hours before experimentation.

A fasting blood sample was withdrawn from a tail vein at the beginning of the experimental period. This was followed by the intraperitoneal injection of a standard dose of glucose (350 mg. per 100 gm. body weight). Two types of glucose solutions were used, 8.75% glucose in water and 8.75% glucose in 0.85% NaCl. The solutions were warmed to 38°C. and injected accurately, being delivered from a 25 ml. pipette graduated in 0.1 ml. The animals were then placed in metabolism cages, and the urine collected during a 5-hour period. At the end of this time another sample of blood was withdrawn from a tail vein and nembutal (3 mg. per 100 gm. body weight) was injected intraperitoneally. In rapid succession the abdomen was opened, the peritoneal fluid quantitatively removed, the urinary bladder punctured, drained and washed, and the liver separated from the carcass and both plunged into a mixture of ether and solid carbon-dioxide. Uninjected controls received a similar treatment.

[3] Stock colony ration: "Calf builder" meal, 91% (Larrowe Milling Co., Detroit); wheat germ, 3%; dried brewers' yeast, 3%; and U.S.P. cod liver oil, 3%.

Methods

Glucose was determined in urine, peritoneal fluid, blood, tissue and glycogen hydrolysates by the method of Hagedorn and Jensen ('23) or the macro-modification of Hanes ('29). The question of non-sugar reducing substances was carefully investigated in all fluids with the exception of urine where the total reducing substances were too small to significantly affect the results of this investigation. Yeast fermentation studies on a number of blood filtrates and peritoneal fluids showed a relatively constant, small amount of non-sugar reducing substances to be present. The averages of these determinations were used to correct all blood and peritoneal fluid sugar values. Non-sugar reducing substances amounted to 14 mg. per 100 ml. of blood and 3.7 mg. per 100 mg. of unabsorbed glucose in the peritoneal fluid. Each individual free sugar and total carbohydrate determination on tissue (liver and carcass) was calculated in terms of fermentable sugar in the filtrate. Glycogen was calculated in terms of the fermentable sugar in the acid hydrolysates of this substance. The sum, tissue free sugar plus tissue glycogen, was practically equal in most instances to tissue total carbohydrate (see table 2 and fig. 1).

Preparation of tissues for analysis

The liver was crushed to a powder in the frozen state by means of a contusion mortar (Graeser, Ginsberg and Friedemann, '34). The powder was thoroughly mixed to insure homogeneity. The rest of the carcass, skin and bones was first broken into small pieces and then minced twice in a grinder. Both the tissue and the grinder were left in contact with "dry ice" during the mincing process. The minced tissue was thoroughly mixed to insure homogeneity and aliquots were taken for the analyses to be described. Determinations on duplicate samples invariably checked. This procedure was adopted after a number of glycogen analyses had been made on individual muscles (gastrocnemii), frozen in situ. The glycogen content of the right gastrocnemius was not in-

variably the same as that of the left. We have been forced to agree with the following statement of Chambers and Barker ('40) concerning analyses on small muscle samples: "A principal objection to these is the practical impossibility of judging the glycogen (or other carbohydrate) content of the entire body musculature from one or two isolated muscle samples."

TABLE 1

Methods of carbohydrate analyses.

ANALYSIS	TISSUE	AMOUNT OF TISSUE	PROTEIN PRECIPITATED BY ADDITION OF			AUTHORITY
			1 N H_2SO_4	10% $ZnSO_4$	2 N NaOH	
		gm.	*ml.*	*ml.*	*ml.*	
"Free" glucose	Liver	2	10.0	6.0	7.3	Blatherwick et al., ('35)
"Free" glucose	Carcass	20	50.0	30.0	34.5	Blatherwick et al., ('35)
Total carbohydrate	Liver	2	20.0	12.0	13.5	Cori and Cori ('33)
Total carbohydrate	Carcass	50	7.5[1]	6.0	5.6	Cori and Cori ('33)
			TISSUE DISSOLVED IN			
Glycogen	Liver	1	4 cc. 30% KOH			Good et al., ('33)
Glycogen	Carcass	20	40 cc. 30% KOH			Good et al., ('33)

[1] Amount present in 10 ml. aliquot of tissue hydrolysate.

Tissue analyses

Table 1 gives the amounts of tissues and reagents used in the various analyses, together with the references to the procedures used. In the liver, where the amount of tissue is limited, duplicate determinations for free sugar and total carbohydrate were made on the filtrate of single 2 gm. samples. Two separate 1 gm. samples of liver were used for glycogen determinations. In the carcass, four 20 gm. and one 50 gm.

aliquots were analyzed. Of the four 20 gm. samples, two were used for free sugar and two for glycogen. The 50 gm. sample used for total carbohydrate determination was hydrolyzed 4 hours in 300 ml. of 1 N H_2SO_4 and then diluted to 400 ml. volume. A pipette with an orifice 1.5 mm. in diameter was used to withdraw two 10 ml. aliquots of the fine suspension of tissue.

Glucose transformed

It is possible to calculate from the two separate sets of data the amount of injected glucose transformed to other substances during the course of the 5-hour period. The value represents the amount of injected glucose unaccounted for in the analyses. The following equations have been used to make the calculation: (1) Total carbohydrate of uninjected control, plus glucose absorbed and retained, minus total carbohydrate of injected animal equals the glucose transformed during the 5-hour period. (2) Glycogen plus free sugar of uninjected controls, plus glucose absorbed and retained, minus glycogen, plus free sugar of injected animal, equals glucose transformed during the 5-hour period. The values calculated from equation (1) agree closely with those calculated from equation (2) (see table 2 and fig.1). It is to be emphasized that the value for glucose transformed, as here defined, not only includes glucose oxidized, but also glucose converted to fat, pyruvic acid, lactic acid and other possible intermediates.

RESULTS

The present investigation is concerned with the effect of sodium chloride upon the characteristic low tolerance to glucose of the Yale strain of rats. The nature of this abnormality has been extensively reported elsewhere (Orten and Sayers, '42), but to facilitate an understanding of the action of NaCl the responses to glucose characteristic of a presumably normal strain (Wistar) have been included in table 2. Any change produced by this salt in the carbohydrate levels of the Yale strain can be compared to the already normal levels of the

DISTRIBUTION OF CARBOHYDRATE	YALE FASTING (6)[1] Mean and S.D.[2]	S.R.[3]	WISTAR FASTING (6) Mean and S.D.	WISTAR GLUCOSE ALONE (9) Mean and S.D.	S.R.	YALE GLUCOSE ALONE (10) Mean and S.D.	S.R.	YALE GLUCOSE + NaCl (9) Mean and S.D.	S.R.[4]
Glucose absorbed mg./100 gm. rat				277 ± 35.6	0.0	277 ± 28.4	0.47	281 ± 26.0	0.40
Glucose excreted mg./100 gm. rat				3 ± 1.5	0.88	4 ± 5.3	2.58	9 ± 7.3	3.70
"True" blood glucose									
Initial mg./100 cc.	77 ± 4.7	5.81	66 ± 10.3						
Final mg./100 cc.				115 ± 25.0	5.19	219 ± 90.4	4.35	125 ± 42.9	0.89
Liver									
"Free" glucose mg./100 gm. rat	2.8 ± .36	4.65	2.1 ± .39	6.4 ± .93	3.89	8.5 ± 2.16	3.26	5.8 ± 2.72	0.88
Glycogen[5] mg./100 gm. rat	10.6 ± 4.65	3.15	5.4 ± 3.79	45.3 ± 21.57	2.37	30.7 ± 17.98	3.21	44.4 ± 8.58	0.18
Total carbohydrate mg./100 gm. rat	17.4 ± 6.72	2.98	10.3 ± 5.50	54.9 ± 19.12	1.96	43.2 ± 19.55	2.80	57.7 ± 13.94	0.49
Carcass									
"Free" glucose mg./100 gm. rat	22 ± 6.5	1.20	19 ± 6.6	42 ± 11.4	4.37	70 ± 24.5	4.40	41 ± 12.2	0.24
Glycogen mg./100 gm. rat	239 ± 68.2	0.58	253 ± 56.6	359 ± 60.8	6.70	234 ± 57.0	6.57	370 ± 68.3	0.52
Total carbohydrate mg./100 gm. rat	285 ± 34.0	1.27	263 ± 53.1	389 ± 48.7	2.26	346 ± 69.4	2.86	400 ± 45.0	0.73
Summary (Carcass and liver)									
Total carbohydrate data									
Total carbohydrate mg./100 gm. rat	302 ± 31.5	1.67	273 ± 55.0	444 ± 47.4	2.52	389 ± 84.7	3.20	458 ± 43.2	0.95
Total carbohydrate in excess of fasting level mg./100 gm. rat				171 ± 47.4	2.52	87 ± 84.7	3.20	156 ± 43.2	0.95
Injected glucose transformed[5] mg./100 gm. rat				103 ± 72.0	3.24	185 ± 86.2	3.28	112 ± 45.5	0.45
"Free" glucose and glycogen data									
"Free" glucose and glycogen, mg./100 gm. rat	274 ± 73.1	0.23	280 ± 60.7	453 ± 51.3	6.08	343 ± 65.2	5.69	461 ± 64.9	0.42
"Free" glucose and glycogen, in excess of fasting level mg./100 gm. rat				173 ± 51.3	5.74	69 ± 65.2	5.67	187 ± 64.9	0.73
Injected glucose transformed,[5] mg./100 gm. rat				101 ± 84.8	4.16	203 ± 68.6	5.15	81 ± 75.4	0.76

[1] Number of animals in parentheses.
[2] S. D. = standard deviation.
[3] S. R. = significance ratio (Sherman, '37) between means immediately to the right and left.
[4] Between Yale strain (glucose + NaCl) and Wistar strain (glucose alone).
[5] All carbohydrate values expressed in terms of glucose.

Wistar strain given glucose alone. From table 2 it is to be noted that there is no significant difference in the peritoneal absorption or renal excretion of the injected glucose in the two strains. In the uninjected controls, the Yale strain animals have significantly higher levels of blood sugar, liver free
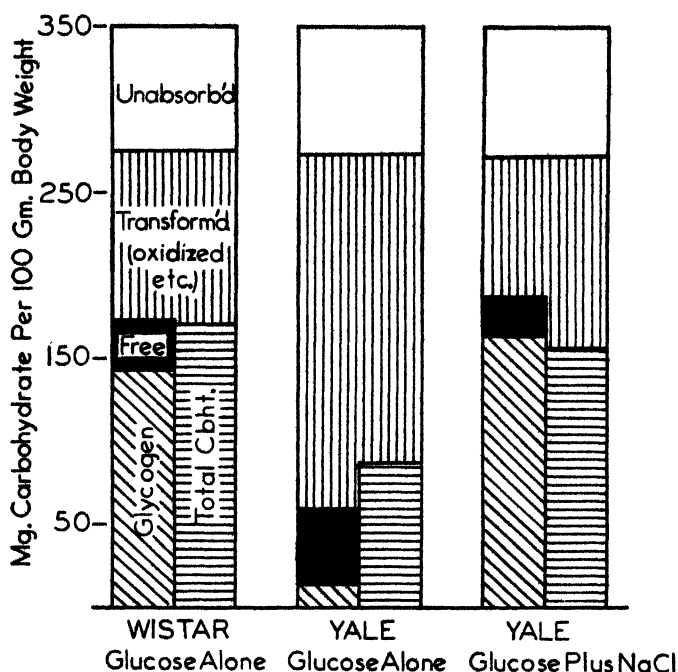

Fig. 1 Summary diagram showing effect of NaCl on the disposition of injected glucose in Yale strain of rats which have a low tolerance to glucose.

sugar, liver glycogen and liver total carbohydrate. No significant difference exists between the carcass values in the fasting state. In the injected animals, however, smaller amounts of glycogen in the liver and remaining carcass are to be found in the Yale strain, together with significantly larger amounts of "free" sugar.

From table 2 it can also be seen that the very slightly higher average level of glucose absorption in the sodium chloride treated animals is not significantly different from that of the

Yale and Wistar strains given glucose alone. The salt does exert a slight glycosuric action, but when the amount of glucose absorbed and retained is calculated it happens that this value is the same in all three groups of animals. When the NaCl is given in a concentration isotonic to the glucose, i. e., 1.58%, the absorption of the glucose is not affected, but a more marked glycosuria takes place whose influence upon the amount of glucose absorbed and retained makes this higher salt concentration unsuitable for a balance study (Sayers and Orten, '41).

Examination of table 2 shows that the fifth hour blood sugar of the "Yale" strain is considerably lowered when the animals are given NaCl with the administered glucose; in fact, the values are essentially normal when compared with those of the Wistar strain. This confirms previous reports from this laboratory (Orten and Devlin, '40), using 1.58% NaCl. It is to be noted that with the weaker concentration of salt a normal tolerance is obtained in a situation where the amount of glucose absorbed and retained is the same in both the "glucose-alone" and the "salt-treated" animals.

Significant differences in tissue carbohydrate levels between the NaCl-treated rats and the Yale strain animals given glucose alone are shown in table 2. In both the liver and carcass, the free sugar is decreased and the glycogen and total carbohydrate are increased in the salt-treated animals. When the significance ratios between the averages of the three groups — the "Wistar" animals given glucose alone, the "Yale" rats given glucose alone, and the "Yale" animals receiving sodium chloride — are examined, it can readily be seen that NaCl brings about the production of normal carbohydrate levels in the otherwise abnormal "Yale" strain rat. Calculation of glucose transformed (oxidized, etc.) shows that this value is reduced to a normal level in the "Yale" strain animals given NaCl. Apparently, the animals are enabled to store enough of the injected glucose to be commensurate with a normal tolerance curve.

All of the above facts are diagrammatically represented in figure 1.

DISCUSSION

The injection of a hypertonic solution of glucose into the peritoneal cavity is followed by the accumulation of electrolytes in this space at the expense of the blood and tissues (Darrow and Yannet, '35). In this connection it should be noted that the "Yale" strain animals, in contrast to those of the Wistar strain, present a picture of irritability, and even in some cases of muscular spasticity beginning with the second or third hour subsequent to the administration of glucose. Sodium chloride-treated rats of the "Yale" strain show none of these symptoms. Five hours after the administration of the glucose, the peritoneal cavity contained a volume of fluid twice as great as the amount injected. Not only was this true of the Wistar and "Yale" animals given glucose alone, but also of the animals given glucose dissolved in sodium chloride of either 1.58% or 0.85% concentration. Neither the strain difference nor the action of sodium chloride influenced the amount of fluid lost by the animal. A strain difference may exist in the amount of sodium chloride passing into the peritoneal cavity. No quantitative studies of electrolyte balance, necessary to settle this problem, have been made.

Concentrations of 0.85% and 1.58% sodium chloride are equally effective in improving the low tolerance to glucose of the "Yale" strain of rats. A factor to be considered in the higher concentration of salt is the loss of glucose by way of the kidney. That this is neither a necessary nor a primary effect of the salt is proven by the fact that 0.85% sodium chloride acts in a situation where the amount of glucose absorbed and retained is the same as in the "glucose alone" treated animals. McQuarrie, Thompson and Anderson ('36) have reported a decreased urinary loss of glucose in certain human diabetics under sodium chloride therapy.

Sodium chloride does not help the utilization of glucose by way of mechanisms involving its transformation (oxidation, etc.); in fact, by calculation (see table 2 and fig. 1) it appears

that less glucose is utilized by this means in the presence of salt. The accompanying reduction in muscular activity after sodium chloride treatment may account for this decrease.

The improved glucose tolerance of the "Yale" strain of rats given sodium chloride is definitely a consequence of the increase in the deposition of administered glucose as glycogen. We have no knowledge of the exact mechanism involved. Lewis and Longwell ('41) found the tolerance to glucose and the sensitivity to subcutaneously administered insulin to be greater in animals maintained on a high sodium chloride diet than in control animals. Their evidence suggests that storage changes rather than alterations in oxidative reactions brought about the observed results. This is in agreement with our findings. Adrenal demedullation as well as NaCl administration result in the production of a normal tolerance in the "Yale" strain of rats (unpublished data). It is possible that NaCl (1) decreases epinephrine secretion, (2) inhibits the glycolytic action of epinephrine, (3) counteracts vascular disturbances incident to hyper-secretion of epinephrine, or (4) operates independently of this hormone.

SUMMARY

The effect of sodium chloride on the disposition of intraperitoneally injected glucose has been studied in a strain of rats having a low tolerance to glucose.

Sodium chloride (0.85%) given with the glucose improves the tolerance of these rats, but has no significant effect upon the peritoneal absorption or renal excretion of the glucose.

The administration of sodium chloride with glucose favors the deposition of glycogen in both the liver and the rest of the carcass, simultaneously decreasing the "free" sugar in the blood and tissues. The transformation (oxidation, conversion to fat, etc.) of glucose also appears to be significantly decreased to a normal level.

It appears that sodium chloride improves the low tolerance to glucose of the "Yale" strain of rats primarily by increasing the storage of the administered glucose as glycogen.

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THE EFFECT OF VITAMIN D ON CALCIUM RETENTIONS [1]

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Although most foods contain little or no vitamin D, the presence of important amounts of this substance has been demonstrated (Sherman, '41) in egg yolk, whole milk and butter fat. According to Park ('39), a large proportion of children and adults must receive a moderate amount of vitamin D in their food. This moderate amount, notably inadequate for the growth needs of infants and children, may be adequate for the adult because of his decreased need. Nevertheless, Park suggests that under certain conditions it is advisable for adults to have some additional source of vitamin D. He states that among those adults who need additional vitamin D are: (1) "those deprived of the opportunity of obtaining the vitamin from the sun, and (2) all persons whose diet is lacking in milk or is generally poor."

Sherman ('41) points out that although there is little evidence as to whether or not adults need vitamin D, it has been shown that vitamin D does not lower the minimum need for calcium. Jeans and Stearns ('39) state that observations on adults indicate, as for children, that the ingestion of vitamin D in no way lessens the requirement for calcium.

Jeans and Stearns ('39) also state that the requirements for vitamin D for the adult who has ample intakes of calcium and phosphorus and a diet otherwise adequate, may be defined as the amount needed for maintenance of bony and dental structures during adult life, and that the most useful criterion of the vitamin D requirement is provided by studies of the utilization of calcium and phosphorus. They further suggest that "because the quantity of phosphorus retained varies with the nitrogen as well as the calcium retention, the quantity of calcium retention may be used as the most convenient guide."

In comparing the calcium, phosphorus and nitrogen retentions of a group of 124 young women who were on their customary self-chosen diets, with the corresponding retentions of a group of nine women who were on well-selected basal diets supplemented daily by 250, 500, and 750 cc. of milk, respectively, at different intervals (McKay et al., '42), it was noted that at corresponding intakes, the women on the controlled diets retained more calcium than those on their customary self-chosen diets. This improved retention was especially noticeable for the subjects who were ingesting less than the recommended allowance of 0.8 gm. calcium daily.

EXPERIMENTAL PROCEDURE

The study here reported is concerned with the possible effects of vitamin D upon retention of calcium when women were using well-selected diets. For the purpose of this investigation, six of the young women who had been subjects in the study of the effect of diet upon retention of calcium continued as subjects in further tests.

The six subjects had previously been on the basal diets supplemented daily by 250, 500, and 750 cc. of milk, respectively, for three 10-day periods, each observation period being preceded by a 15-day foreperiod. Foods included in the basal diet were similar in kind and amount for each subject (table 1). To provide for varying calorie needs, certain foods of negligible mineral content, such as butter, mayonnaise, French dressing, jelly, cookies, white bread and macaroni, were used ad libitum by the subjects.

Upon completion of the last of three observation periods, at which time the diet was supplemented by 750 cc. of milk, the subjects were returned to the basal diet supplemented by 250 cc. of milk. They continued on this dietary regimen until the calcium retentions were approximately the same as they had been during the previous periods when similar diets supplemented by 250 cc. of milk had been used.

At this point, to test the possible effect of supplemental vitamin D upon retentions, each subject was given daily approximately 500 I.U. of vitamin D.[2] At the conclusion of a 15-day

TABLE 1

Basal diet used during each 5-day period.

FOOD	AMT.	FOOD	AMT.	FOOD	AMT.
	gm.		gm.		gm.
Beef	400	Orange juice	200	String beans	70
Bacon	20	Grapefruit	100	Beets	70
Salmon	90	Tomato juice	200	Cabbage	100
Egg	150	Tomato	100	Carrots	100
Whole wheat bread	375	Banana	100	Lettuce	100
		Canned peaches ...	100	Peas	70
		Canned pineapple..	80	Potatoes	400
				Potato sweet	100

foreperiod, there were two consecutive 5-day collection periods. This routine was followed for milk intake levels of 250, 500, and 750 cc., respectively. One subject was obliged to drop out of the study at the end of the first vitamin D period. This left a group of five women for the two remaining vitamin D studies.

Methods of collection, sampling, and analysis were similar to those described in the preceding study (McKay et al., '42). Six 10-day observation periods for each of the five subjects and two 10-day periods for the sixth subject are reported. Of the thirty-two 10-day studies, twelve were made in Kansas and twenty in Ohio.

RESULTS AND DISCUSSION

Table 2 summarizes data for the studies of women on corresponding calcium intakes with and without the vitamin D

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[2] The vitamin D was in the form of viosterol capsules, provided by Mead, Johnson and Company, Evansville, Indiana.

supplement. At mean daily intakes of from 0.5 to 0.8 gm., amounts which were less than the daily allowance for women recommended by the Committee on Food and Nutrition (now Food and Nutrition Board) of the National Research Council ('41) the mean retention figure (0.013) for the six individuals who were using vitamin D in addition to the well-selected controlled diets was somewhat less than the corresponding figure (0.029) for those individuals who were having no vitamin D.

TABLE 2

Calcium metabolism data for forty-two studies of women on controlled diets (in grams).

SERIES I. NO ADDITIONAL VITAMIN D			SERIES II. ADDITIONAL VITAMIN D		
Nine individuals with 26 studies [1]			Six individuals with 16 studies		
No. of studies	Mean intakes	Mean balances	No. of studies	Mean intakes	Mean balances
Mean Daily Intake Range 0.500–0.799 gm.					
3	0.560	0.019	2	0.587	0.030
5	0.648	0.035	4	0.611	0.000
2	0.765	0.028	..	....	....
Mean	0.645	0.029		0.603	0.013
Mean Daily Intake Range 0.800–1.299 gm.					
3	0.809	— 0.061	3	0.886	0.082
2	0.940	0.067	2	0.918	0.112
3	1.061	0.133	1	1.094	0.103
5	1.147	0.104	3	1.167	0.103
3	1.264	0.076	1	1.200	0.158
Mean	1.064	0.069		1.029	0.104

[1] Compiled from data previously published (McKay et al., '42).

That this difference was not significant statistically was shown by application of Student's ('25) t test to the difference between the mean retentions adjusted to a mean intake (P = 0.72). The use of approximately 500 I.U. of vitamin D had no influence upon the calcium retentions of the six individuals who were ingesting from 0.500 to 0.799 gm. calcium daily. These data corroborate the conclusions of Sherman ('41) and of Jeans and Stearns ('39) that the use of vitamin D does not lower the minimum need for calcium.

TABLE 3

Calcium intakes and retentions or losses for five individuals (in grams).

BASAL DIET SUPPLEMENTED BY:	PERIOD	O. M. (KAN.)		B. W. (KAS.)		E. M. (OHIO)		F. H. (OHIO)		D. M. (OHIO)	
		Intake	Retention	Intake	Retention	Intake	Retention	Intake	Retention	Intake	Retention
250 cc. milk	I a										
1st 5 days		0.662	0.175	0.618	0.100	0.572	0.166	0.564	0.118	0.559	0.075
2nd 5 days		0.657	—0.099	0.610	0.100	0.542	—0.069	0.651	0.077	0.575	—0.059
Mean		0.660	0.038	0.614	0.100	0.557	0.048	0.608	0.098	0.567	0.008
Plus 500 I.U. vit. D	I b										
1st 5 days		0.673	0.171	0.614	0.020	0.591	0.026	0.594	0.058	0.602	—0.175
2nd 5 days		0.558	0.050	0.563	0.009	0.627	0.144	0.576	0.031	0.632	—0.062
Mean		0.616	0.111	0.589	0.015	0.609	0.085	0.585	0.044	0.617	—0.118
500 cc. milk	II a										
1st 5 days		1.026	—0.083	1.027	0.171	0.819	0.168	0.817	0.092	0.786	—0.132
2nd 5 days		0.830	—0.012	0.874	0.048	0.774	0.071	0.824	—0.119	0.831	—0.155
Mean		0.928	—0.048	0.951	0.110	0.796	0.120	0.820	—0.014	0.808	—0.144
Plus 500 I.U. vit. D	II b										
1st 5 days		0.934	—0.058	0.903	0.143	0.886	0.053	0.921	0.116	0.913	—0.015
2nd 5 days		0.893	0.196	0.857	0.118	0.878	0.098	0.923	0.079	0.878	0.096
Mean		0.914	0.127	0.880	0.131	0.882	0.076	0.922	0.098	0.896	0.040
750 cc. milk	III a										
1st 5 days		1.355	—0.081	1.067	0.130	1.132	0.080	1.174	0.156	1.126	—0.139
2nd 5 days		1.219	0.235	1.187	0.119	1.151	0.019	1.133	0.231	1.171	0.161
Mean		1.287	0.077	1.127	0.125	1.142	0.050	1.154	0.194	1.148	0.011
Plus 500 I.U. vit. D	III b										
1st 5 days		1.179	0.164	1.109	0.067	1.174	0.209	1.114	0.128	1.154	—0.038
2nd 5 days		1.147	0.195	1.078	0.138	1.227	0.108	1.190	0.017	1.218	0.149
Mean		1.163	0.180	1.094	0.103	1.200	0.158	1.152	0.072	1.186	0.056

At intake levels of 0.8 gm. and above, evidence of slightly better utilization of calcium by the women on the basal diets supplemented by vitamin D was shown by the mean retention figure 0.104 as compared with 0.069. When these retention figures were adjusted to a mean intake and Student's t test applied to the difference, the result indicated some influence of the additional vitamin D but not enough to be significant (P = 0.26).

Although mean figures for calcium retentions showed no significant difference due to the use of vitamin D, there was considerable variation in individual reactions (table 3). For two women, B. W. and F. H., the total calcium retention for the three periods of vitamin D intake was less than when no supplemental vitamin D was used. Calcium utilization was therefore fully as efficient either with or without supplemental vitamin D. For D. M. and E. M., the evidence of improved retention with the use of vitamin D was inconclusive. The fifth subject, O. M., retained more calcium during each of the vitamin D periods than during corresponding periods when no vitamin D was given, total calcium retention without vitamin D being 0.067 gm. as compared to 0.418 gm. when vitamin D was used. When the Student's t test was applied, however, the difference between the two retentions was not found to be significant (P = 0.15).

It is sometimes assumed that the distribution of phosphorus and calcium excretion changes when vitamin D is given. No such change took place in the case of these young adults, the percentage of both calcium and phosphorus excreted in the urine being little changed as the result of the use of additional vitamin D.

SUMMARY AND CONCLUSIONS

Six young college women were given a well-selected basal diet supplemented daily by 250, 500, and 750 cc. of milk, respectively, for each of three collection periods of 10 days each. Subsequently, corresponding diets supplemented by approximately 500 I.U. of vitamin D were given to one of the subjects

for one 10-day period and to the other five subjects for three 10-day periods each. At mean daily calcium intake levels of less than 0.8 gm., no evidence was shown of improved calcium retention with vitamin D. At mean daily calcium intake levels of 0.8 gm. and above, there was some evidence of improved retention with vitamin D, but the improvement was so slight that it was not statistically significant.

Although for one of the five subjects the mean retention figures improved during each of the vitamin D periods, the difference between the two mean retention figures was not significant.

It is concluded that the addition of approximately 500 I.U. of vitamin D to diets which were well-selected had little influence upon the calcium retention, and that the well-selected diet provides for the vitamin D needs of the young adult.

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THE EFFECTS OF PANTOTHENIC ACID AND INOSITOL ADDED TO WHOLE WHEAT BREAD ON EVACUATION TIME, DIGESTION AND ABSORPTION IN THE UPPER GASTRO-INTESTINAL TRACT OF DOGS [1]

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TWO FIGURES

INTRODUCTION

In view of the recognized importance of the B-complex vitamins on gastrointestinal function, and on the basis of the favorable results on carbohydrate digestion and absorption with pantothenic acid indicated by Russell and Nasset ('41), it was felt that an expanded study should be made of the effect of this and other factors on the digestive tract. While McKibbin et al. ('40) and Schaefer et al. ('42) have mentioned alimentary canal changes in pantothenic acid deficiency, no report has included an extended study on the chronic effect of pantothenic acid deficiency on gastrointestinal function.

Our object was to study, in jejunostomized dogs, a chronic pantothenic acid deficiency with regard to gastrointestinal motility as well as carbohydrate and protein digestion and absorption.

EXPERIMENTAL

The procedures employed were similar to those outlined by Russell and Nasset ('41) and Lambooy and Nasset ('43). In adult mongrel dogs, Maydl jejunostomies were established

approximately 50 cm. caudad from the ligament of Treitz. At least 12 hours after last food (20 hours in the deficiency periods), the dogs were placed in a standing position in a stall and supported so as to provide the least possible discomfort. A two-lumened catheter with a rubber balloon on the end was introduced through the fistula well into the lumen of the gut. When the catheter was in place, the balloon was inflated through the small lumen with approximately 10 cc. of air, thus minimizing the loss of food past the catheter. Through the larger lumen of the catheter, the chyme, as it passed the enterostomy, was drawn by a negative pressure of 12 cm. of water into an appropriate receiving vessel.

After a pre-feeding collection period of at least $\frac{1}{2}$ hour to make certain that the intestine was free of food, a weighed, analyzed test meal was fed. Collections of the chyme from the receiving vessel were made at approximately half-hour intervals, weighed, and the pH determined. The collections were then placed in a boiling water bath for 15 minutes to destroy all enzyme activity, pooled for each experiment, and stored at 5°C. until the analyses were made.

The following three methods were used to determine when the end of the experiment had been reached; in other words, to insure that the total emptying time required for complete passage of the test meal from the mouth to the catheter had elapsed: (1) the occurrence of a negative Benedict's qualitative sugar test on the collection; (2) the low weights of the half-hour collections; (3) the increase of pH occurring toward the last part of the experiment. The runs were continued for an additional hour or more to make certain that the intestine was empty. Since the emptying time and gastrointestinal motility must bear a fairly close, inverse relationship to each other, the former may be taken as a measure of the latter.

The basal diet which dog 1 received to establish a normal control consisted of water- and alcohol-extracted casein, 25%; alcohol-extracted sucrose, 41%; hydrogenated vegetable oil,[2]

[2] Primex.

18%; bone ash, 4%; dried brewers' yeast, 10%; and salts, 2%. The salts were those of the Phillip salt mixture as modified by Arnold and Elvehjem ('39), plus cobalt according to Frost et al. ('41).

The experimental diet consisted of a whole wheat bread made from flour prepared by the Earle flotation process. This bread was obtained fresh daily and fed moistened with water, about 400 gm. of bread being consumed. No difficulty was experienced in getting the dogs to eat the bread, and no dislike for it was ever evidenced, even after several months. The composition of the diet is given in table 1, the vitamin content having been determined by Sealock and Livermore ('43).

TABLE 1

Composition of experimental diet.

BREAD AS EATEN	VITAMIN CONTENT
	(μg./gm. fresh bread)
N 1.67% (protein 9.63%)	Thiamine — 2.73 (fermentation method)
	Riboflavin — 2.45 (rat growth)
Carbohydrate (by hydrolysis) 38.4%	Pyridoxine — 3.14 (rat growth)
	Pantothenic acid — 5.2 (bacterial growth)
	Inositol — 644.0 (yeast growth)
	Niacin — 30.0 (bacterial growth)

The dogs were fed the diet plus any vitamin supplements six times per week and always on the day preceding an experiment. Any dog suspected of coprophagy was muzzled in such a way as to prevent this, yet to permit the dog to drink water, which was provided ad libitum. The general program of feeding was to produce the deficiency, add the pantothenate supplement, reproduce the deficiency by removing the supplement, repeat the test of pantothenate supplement, and then testing the effects of other factors, including pyridoxine and inositol. The periods of deficiency and supplementation were alternated several times to permit detection of progressive changes.

In all periods the diets were supplemented with adequate amounts of the oil soluble vitamins A, D, E, and K, as follows: A, 200 I.U./kg./day; D, 10 I.U./kg./day; E, 1 mg./kg./day; K, 1 mg./kg./day.

The test meal consisted of 100 gm. of thoroughly dried and ground experimental diet, and was fed with 250 cc. of water at the beginning of each experiment, after the pre-feeding collection had been made. If a dog on any day refused to eat the entire test meal or if, for any reason, such as undue excitement or restlessness, the dog was considered not to be in a normal state, the experiment was discarded.

Analyses were made both for reducing sugar and total carbohydrate by the Shaffer-Somogyi ('33) method, and for diffusible and total nitrogen by the Kjeldahl method. From these data and from analysis of the test meal the amounts of carbohydrate and protein which had been digested and absorbed were calculated.

<div align="center">RESULTS</div>

1. Motility

It was found that the gastrointestinal motility decreased in two dogs maintained on the whole wheat bread diet supplemented only with the oil-soluble vitamins, and that additional pantothenic acid was necessary for the proper maintenance of that motility.

Dog 1 showed an average emptying time of 5.9 hours for the basal period of 4 weeks on the complete basal diet (fig. 1). When the animal was fed the experimental diet, the emptying time gradually increased in 7 weeks to an average of 11.8 hours. The administration of calcium pantothenate[3] at a level of 220 µg. per kilogram of body weight per day was begun after 9 weeks on the experimental diet. During the week following the beginning of administration of the vitamin, the emptying time decreased from an average of 11.8 hours (three experiments) to a normal average of 5.0 hours (two experiments). Using Fisher's ('38) t test the probability of this difference occurring by chance was calculated to be less than 1 in 100. Unfortunately, this animal later required a repair

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[3] Kindly supplied by Merck and Company.

operation due to a closing fistula and died post-operatively. Death was not ascribed to the deficiency.

Dog 2, having been on the experimental diet nearly a month before experiments were begun, was already slightly deficient. The emptying time for the next 3 weeks increased to an
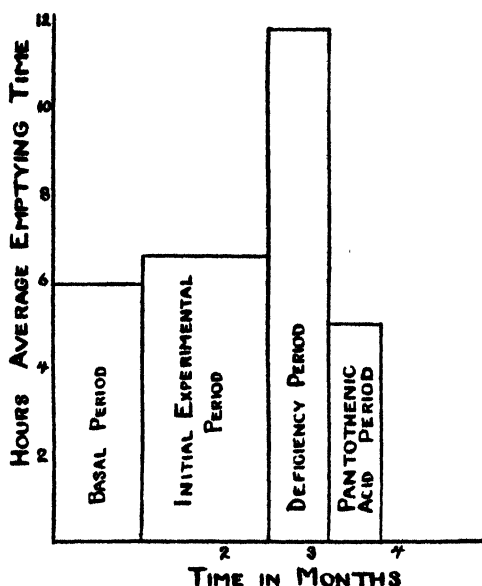
Fig. 1   Average emptying times for dog 1.

average of 10.4 hours (fig. 2). Within a week of calcium pantothenate administration at the same level as for dog 1, the emptying time dropped to a normal average of 5.6 hours over a period of a month. Upon removal of the pantothenate supplement the emptying time again gradually increased, reaching an average of 12.2 hours in 2½ months. Administration of the pantothenic acid at two later periods when the dog was in the deficient state resulted in each case in a prompt return of the motility to normal. On the basis of these combined periods the average emptying time during the deficiency periods was 11.5 hours (fourteen experiments) as compared to 6.4 hours for the vitamin control periods (nine experi-

ments). Again the calculated probability of chance was less than 1 in 100. Hence, a deficiency of pantothenic acid in one period with dog 1 and in three different periods with dog 2 resulted in an average increase in emptying time above normal of 100% and 80%, respectively (figs. 1 and 2). Addition of 220 µg. of calcium pantothenate per kilogram per day restored the motility to normal. It appears likely, therefore, that the adult dog's daily pantothenic acid requirement lies somewhere
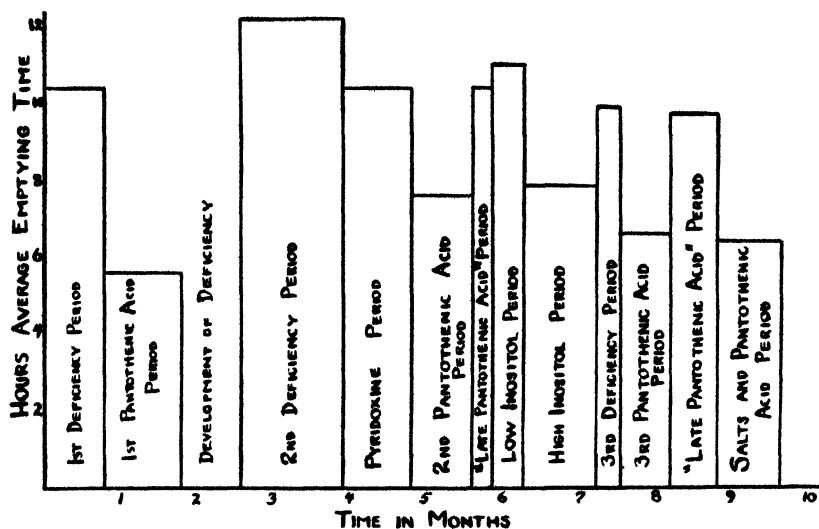
Fig. 2 Average emptying times for dog 2.

between 130 µg./kg. of body weight, the amount supplied by the bread, and 350 µg./kg. of body weight.

During pyridoxine administration (40 µg./kg./day) for a period of 1 month no significant effect on the deficient G. I. functions could be demonstrated, the average emptying time for the period being 10.4 hours for four completed runs, or 60% over the basal level. In three other attempted runs, the dog vomited, having already retained the test meal in the stomach (very small collections, if any, through the catheter) for 7–10 hours after feeding. During this period healings of a lesion on one leg and of a lesion in the mouth were noted,

though these lesions had not been ascribed to a pyridoxine deficiency.

Martin et al. ('41) mentioned constipation in inositol-deficient mice, and reported that in dogs on a constipating though nutritionally adequate diet, inositol, as studied roentgenologically, had a marked stimulatory effect on intestinal peristalsis. Anderson ('16) had noted that inositol dosing caused a transitory diarrhea in man and dogs. Woolley ('42) has reported the intestinal synthesis of inositol in mice. He also reported that in vitro synthesis by intestinal flora did not proceed in the absence of pantothenic acid, and again ('41) that when sufficiently high levels of pantothenic acid were fed, alopecia frequently did not appear in inositol-deficient mice. Hence, it was thought that inositol might be tried to determine whether or not pantothenic acid acted indirectly by stimulating inositol production.

Subcutaneous administration of inositol (up to 33 mg./day) had no effect on the reduced intestinal motility (fig. 2), as would be expected with the large (though partially unavailable as phytin) content of inositol already present in the bread diet (table 1). Subsequent oral administration of up to 500 mg. per day of inositol resulted in reduction in the emptying time, one experiment being only 4½ hours long. However, as Anderson had noted with a normal diet, the effect was transitory and the length of the time again increased to over 10 hours in 2 weeks.

After 5 to 6 months on the bread diet, dog 2 began to show the effects of a secondary deficiency. After 3 weeks or so of the pantothenic acid supplementation, the emptying time tended to increase, despite continued administration of the vitamin. Increasing the dosage of the vitamin had no effect on this tendency of the "late pantothenic acid" runs. A complicating salt deficiency seemed probable; hence, 8 gm. per day of the basal salt mixture was added to the diet of dog 2 which was already receiving 3.30 mg. of pantothenic acid per day. Within a few days the emptying time decreased to an average of 6.2 hours (five experiments) — a value nearly

identical with the early value for pantothenic acid alone (fig. 2). It was concluded that there had been a complicating salt deficiency which had grown more and more severe over the months the animal was kept on the experimental diet.

Other than the effect on motility, there were interesting and possibly significant results. During the first deficiency period for each dog, as the emptying time increased, the normal sweet smell of chyme was replaced by a foul odor identified as that of hydrogen sulfide. This was not due to back seepage of gas from the colon of the atonic gut because, upon standing, the concentration of the gas in a given collection markedly increased. The odor of the H_2S disappeared during the periods of pantothenic acid feeding, but reappeared in dog 2 when the vitamin supplement was omitted from the diet. The muzzle worn by the dog eliminated the possibility of coprophagy. From twelve deficiency experiments, an average of 0.4 mg. of H_2S per experiment was obtained by the use of an aeration train. Pyridoxine was ineffective in reducing the amount of H_2S produced.

Other observations tended to fit in with a general gastrointestinal atony. During the deficient periods, the "buttons" of the fistulae became notably flabby and atonic, and leaks from the fistulae developed.

2. Digestion and absorption of carbohydrate and protein (see table 2)

It was found, in general, that the average total amounts of carbohydrate and protein digestion and absorption stayed remarkably constant during the different periods, the exceptions being the periods of the high inositol supplementation and of the salts and pantothenic acid supplementation. Marked differences in the rates were found, however, calculations being based for dog 1 on the eight basal runs, and for dog 2 on the nine runs of early pantothenic acid supplementation.

In both dogs, the average rates of carbohydrate and protein digestion and absorption show marked and statistically significant decreases during pantothenic acid deficiency. In dog 1

the digestion rates dropped to only one-half of the normal, while the absorption rates dropped to less than half. The probability of this difference being due purely to chance is less than 1 in 100. The data for dog 2 show similar differences, though not quite as great, for the rates of digestion and absorption between the average of the three different periods of pantothenic acid deficiency and that for the three corresponding periods of pantothenic acid supplementation (figs. 1 and 2, table 2). The differences in rate between basal and pantothenic acid periods in dog 1 are not significant. Pyridoxine supplementation did not alter the course of the deficiency.

Inositol exhibited a marked influence on the rates of carbohydrate and protein digestion and absorption. Relatively small doses (up to 33 mg./day) had no significant effects. Large oral doses (up to 500 mg./day or 32 mg./kg./day), however, exerted marked effects. The rate of carbohydrate digestion dropped to 58%, and that of protein digestion to 44% of the average rate in the pantothenic acid period; in each case this was lower than the rates of the deficiency periods. The average rates of absorption dropped much more, that of carbohydrate to 37% and that of protein to only 17% of the rate in the period on pantothenic acid supplement, both obviously highly significant decreases.

The irritating effect of inositol on the G. I. tract seems to be well-illustrated here, although an attempt was made to minimize the cathartic action by giving the inositol only with the regular daily meal and not with the test meal. A study of the protein absorption reveals this effect most clearly. The very low average total absorption and rate of absorption, coupled with the fact that two of the four analyzed runs showed rates of — .3%/hour and — 1.2%/hour absorption, respectively, indicate marked stimulation of secretion, and perhaps diminished capacity to absorb water, resulting in the recovery of more nitrogen than had been ingested. This seems confirmed by the significantly larger total collection volumes

TABLE 2

Results on digestion and absorption.

DOG NO. AND PERIOD	NO. OF RUNS	LENGTHS (HRS.) OF EMPTYING TIMES	CARBOHYDRATE Digestion Total (gms.)	%/hour	% from basal	p ('Fisher')	CARBOHYDRATE Absorption Total (gms.)	%/hour	% from basal	p ('Fisher')	PROTEIN Digestion Total (gms. N)	%/hour	% from basal	p ('Fisher')	PROTEIN Absorption Total (gms. N)	%/hour	% from basal	p ('Fisher')
Number 1																		
Basal[1]	8	5.9	30.8	10.1	....	...	35.5	10.4	....	...	2.16	14.3	....	...	1.04	6.9	....	...
Deficiency	3	11.8	34.0	5.2	−48	.01	33.4	4.4	−58	.01	2.20	7.1	−50	.01	.98	3.1	−55	.01
Pantothenic acid supplement	2	5.0	33.7	13.0	+29	.2	29.5	9.9	−5	.8	2.28	17.6	+23	.3	.94	7.2	+4	.8
Number 2																		
Pantothenic acid supplement[2]	9	6.4	36.4	9.8	....	...	34.7	8.4	....	...	1.96	12.3	....	...	1.08	6.7	....	...
Deficiency	14	11.5	37.9	6.2	−37	.01	37.6	5.4	−36	.01	1.90	6.5	−47	.01	1.00	3.4	−49	.01
Pyridoxine supplement	4	10.4	36.4	6.5	−34	.02	38.2	6.3	−25	.05	1.81	6.8	−45	.01	.99	3.5	−48	.05
Inositol (small am'ts.) suppl.	4	11.0	40.0	7.1	−28	.05	35.7	6.3	−25	.05	1.65	6.4	−48	.01	.96	3.7	−45	.07
Inositol (large am'ts.) suppl.	4	7.9	26.4	5.7	−42	.01	16.9	3.1	−63	.01	1.06	5.4	−56	.01	.24	1.1	−83	.01
Number 2 "Late pantothenic acid" runs	6	9.7	35.2	6.2	−37	.01	31.4	5.1	−39	.01	1.61	6.4	−48	.01	.96	3.4	−49	.02
Salts supplement	5	6.1	31.9	8.8	−10	.1	23.2	5.9	−30	.05	1.27	8.1	−34	.05	.38	2.4	−64	.01

[1] Exclusive of this period, experimental diet was used in the daily feeding. Experimental test meals were used in all the runs.

[2] 220 µg./kg./day.

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(table 3). Wasteneys and co-workers ('41) have briefly discussed the difficulty of studying the true or absolute digestion and absorption of an ingested protein. It is of interest to note (table 3) that the ratio of total recovered nitrogen to the total collection volume remains relatively constant, even with inositol, showing a practically linear relationship throughout. Since digestive secretions contain little or no reducing substance, no complication from this cause enters into the interpretation of the data for carbohydrate.

TABLE 8

Average total collection volume and recovered nitrogen.

EXPERIMENT		TOTAL NITROGEN ABSORPTION		TOTAL COLLECTION VOLUME	NITROGEN RECOVERED IN COLLECTION
Type	Number				
		gm.	*%/hr.*	*cc.*	*mg./cc.*
Pantothenic acid supplement	9	1.08	6 7	450	3.6
Deficiency	14	1.00	3.4	500	3.3
Inositol	4	.24	1.1	705	3.2

Paradoxically enough, the dog lost no weight during the month of inositol supplementation and the accompanying poor absorption but rather showed a slight gain, the average weight during this period being 16.3 kg., and during the other periods, 15.7 kg. This suggests higher, compensatory absorption of the daily diet in the lower jejunum and ileum.

The whole response to inositol does not appear to be that of the relief of a deficiency of that substance, but rather, of a normal effect superimposed upon an unrelated deficiency.

In the "late pantothenic acid" runs discussed with regard to motility, the rates of digestion and absorption dropped back towards deficiency levels again, showing the existence of a long progressive secondary deficiency. Addition of salts did not relieve this deficiency, although the motility returned promptly to normal. Though the rates of carbohydrate digestion and absorption and protein digestion showed slight improvement, the total amounts of carbohydrate and protein digested and absorbed were lower, especially in the case of protein absorption.

CONCLUSIONS

In enterostomized dogs maintained exclusively on a peeled whole wheat bread diet, a severe deficiency was produced in the course of 2 to 3 months. This was characterized by an approximately 50% decrease in gastrointestinal motility, accompanied by 40–60% decreases in the rates of carbohydrate and protein digestion and absorption. The almost immediate effect of adding a supplement of 220 µg. of calcium pantothenate per kilogram of dog weight per day to this diet was the return of these functions to normal in every case. The total digestion and absorption remained practically the same in the normal deficient states.

Pyridoxine was ineffective in altering the course of the deficiency.

Inositol acted more as a cathartic, its effect being apparently superimposed on the deficiency. While the motility was temporarily improved, the total amounts and rates of digestion and absorption were greatly decreased. A suggestion of synergism between inositol and pantothenic acid was not confirmed by this method.

After 5 to 6 months on the experimental diet, a secondary deficiency was noticed, characterized by diminished effectiveness of continued pantothenic acid supplementation to maintain the normal functions of motility, digestion and absorption. Adding a salt supplement, together with the continued pantothenic acid supplement, resulted in a prompt return of the motility to normal but only in very slight improvements in digestion and absorption rates.

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FURTHER CONSIDERATION OF THE EFFECT OF ALTITUDE ON BASAL METABOLISM

A STUDY ON YOUNG WOMEN RESIDENTS OF DENVER [1]

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ONE FIGURE

The determination of the effect of altitude on the basal metabolism of human subjects has been the object of numerous investigations. The usual procedure has been to determine the basal metabolism on the same subjects at one or more different altitudes. However, in a few instances this problem has been studied by comparing the results obtained on residents of one altitude with those of similar investigations at other altitudes. Thus, McKittrick ('36) has obtained higher values for the basal metabolism of young women residents of Laramie, Wyoming (altitude 7,148 feet) than those reported in certain other similar investigations at lower altitudes. Moreover, McCrery and Wolf ('38) and McCrery, Wolf and Bavousett ('40) have claimed that the basal metabolism of young women of Lubbock, Texas (altitude 3,200 feet) lies between the values found by McKittrick and those reported for elevations below 1,000 feet. The workers at both altitudes have concluded that their respective data indicate an influence of altitude on basal metabolism. On the other hand, Lewis, Iliff, Duval and Kinsman ('43) have found that the basal

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[1] This report is taken from the dissertation submitted by Alberta Iliff to the Faculty of the Graduate School of the University of Colorado, June, 1942, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

metabolism of the same subjects determined at widely different elevations (910 feet, 5,280 feet and 8,720 feet) by the same workers with the same portable closed circuit apparatus (a no. M 84 McKesson Metabolor) does not change significantly as a result of altitude. On account of the divergence between these results and those reported by McKittrick and by Mc-Crery and co-workers, further consideration of this problem seems desirable. Accordingly, unpublished data on the basal metabolism of young women residents of Denver are herewith presented and compared with those of like observations obtained at different altitudes by other investigators.

EXPERIMENTAL PROCEDURE

During the past several years, ninety determinations of basal metabolism have been made on forty-three healthy women between the ages of 17 and 26 years, inclusive, who had been residing in Denver (altitude 5,280 feet) for at least 1 year before the observations were made. The subjects had fasted for a period of from 12 to 15 hours and had rested quietly for 45 minutes immediately prior to the determinations, which were made either with a closed circuit bedside type of apparatus, a no. M 84 McKesson Metabolor, or by the open circuit chamber procedure of Higgins and Bates ('30). The latter method has been used in the metabolism laboratory of the Child Research Council for the past 11 years and has been described in detail by Lewis, Kinsman and Iliff ('37). The basal metabolism of each subject was determined on at least 2 different days. In the seven instances in which the same individual was studied at different ages, the determinations are reported separately for each age just as if they had been obtained on different subjects.

Alcohol check determinations were made at intervals during the study on both the open circuit and the closed circuit apparatus. The values obtained for oxygen consumption varied from the theoretical by $\pm 2\%$ with the chamber procedure, and by $\pm 3\%$ with the closed circuit method.

TABLE 1

Individual data on basal metabolism.

SUBJECT	NUMBER OF TESTS	AGE	WEIGHT	HEIGHT	SURFACE AREA	CAL./24 HRS.	CAL./HR./SQ. M.	CAL./HR./KG.	CAL./HR./CM.
		years	*kg.*	*. m.*	*q. m*				
1	1	17	65.0	160.9	1.680	1313	32.6	0.842	0.340
2 [1]	1	17	60.0	173.3	1.720	1375	33.3	0.955	0.330
3 [1]	1	17	54.4	173.6	1.650	1391	35.1	1.07	0.334
4 [1]	1	17	65.0	168.8	1.745	1432	34.2	0.918	0.353
5	2	18	54.6	168.4	1.620	1311	33.8	1.00	0.324
6	2	18	50.8	156.8	1.490	1297	36.3	1.06	0.344
1	1	18	63.6	161.7	1.670	1299	32.4	0.851	0.335
7	2	18	48.5	167.8	1.530	1204	32.8	1.04	0.299
8	2	19	65.4	170.9	1.765	1361	32.2	0.867	0.332
9	2	19	50.8	160.8	1.515	1230	33.8	1.01	0.319
10	2	19	37.2	150.4	1.265	1098	36.2	1.23	0.304
11	2	19	54.2	172.6	1.640	1276	32.4	0.980	0.308
12	2	20	50.4	176.7	1.620	1330	34.3	1.10	0.314
13	2	20	58.4	167.8	1.660	1322	33.2	0.944	0.328
14	2	20	52.0	175.7	1.630	1282	32.7	1.02	0.304
15	2	20	51.6	165.1	1.560	1321	35.4	1.06	0.334
16	3	21	53.1	168.6	1.600	1130	29.4	0.886	0.279
17	2	21	38.4	149.6	1.275	1066	34.9	1.16	0.279
18	2	21	39.0	148.3	1.275	1087	35.6	1.16	0.306
19	2	21	62.4	172.9	1.740	1364	32.6	0.912	0.329
20	2	22	51.6	162.8	1.540	1138	30.8	0.918	0.292
21	1	22	70.3	186.9	1.940	1443	31.0	0.855	0.322
22	2	22	61.8	175.2	1.755	1136	27.0	0.766	0.270
23	2	22	50.2	167.2	1.550	1173	31.5	0.974	0.292
24	2	23	61.6	175.6	1.750	1234	29.3	0.834	0.292
25	2	23	57.4	160.5	1.595	1187	31.0	0.862	0.308
26	2	23	48.2	162.0	1.490	1190	33.3	1.03	0.306
27	2	23	64.8	167.8	1.735	1366	32.8	0.878	0.340
28	1	23	61.0	170.8	1.715	1256	30.5	0.858	0.306
28	1	24	58.7	170.4	1.680	1186	29.4	0.842	0.290
21	3	24	71.5	186.9	1.950	1419	30.3	0.827	0.317
29	2	24	48.6	166.2	1.520	1097	30.0	0.940	0.275
30	3	24	52.7	169.8	1.605	1169	30.4	0.924	0.287
31	1	24	50.3	158.4	1.490	1048	29.3	0.868	0.276
32	2	24	51.2	163.2	1.535	1160	31.4	0.944	0.296
33	2	24	51.7	167.4	1.570	1172	31.0	0.944	0.292
34	2	24	55.3	177.1	1.685	1266	31.4	0.954	0.298
35	3	24	45.3	165.8	1.475	1169	33.1	1.08	0.293
35	1	25	44.2	165.8	1.455	1085	31.1	1.02	0.273
29	1	25	48.0	166.8	1.520	1073	29.4	0.932	0.268
31	1	25	51.9	158.2	1.510	1043	28.8	0.838	0.275
36	1	25	54.2	166.7	1.600	1167	30.4	0.897	0.292
37	2	25	67.2	161.7	1.710	1256	30.6	0.780	0.324
38 [1]	1	26	45.8	159.0	1.435	1111	32.3	1.01	0.291
36	1	26	54.3	166.9	1.605	1146	29.8	0.879	0.286
39	3	26	59.9	173.7	1.720	1326	32.1	0.922	0.318
40	2	26	54.8	167.9	1.615	1116	28.8	0.849	0.277
41	2	26	59.2	172.6	1.700	1218	29.8	0.858	0.294
42	2	26	54.2	165.4	1.590	1152	32.8	0.962	0.316
43	2	26	57.8	170.9	1.670	1193	29.8	0.860	0.291

RESULTS

The basal metabolism expressed as calories per 24 hours, calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter for each subject is given in table 1. This table also gives the age, weight, height and surface area of each subject. The results are listed in the order of the age attained at the last birthday. When two or more determinations were obtained on the same individual at the same age, the mean values for this age are reported. The surface area is taken from the nomogram of Wilson and Wilson ('20) which is based on the height-weight formula of Du Bois and Du Bois ('16). Since it has been found (Lewis, Iliff and Duval, '43) that the closed circuit apparatus gives results which agree closely with those obtained with the chamber procedure, no designation has been made as to which type of apparatus was used with each subject.

The means, standard deviations and coefficients of variation for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter, respectively, have been calculated for the young women residents of Denver for each half of the age range studied, namely, from 17 to 21 years, inclusive, and from 22 to 26 years, inclusive, and for the entire range of from 17 to 26 years, inclusive. These values are reported in table 2. As would be expected, since these three

TABLE 2

The mean, standard deviation and coefficient of variation for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter obtained on the young women residents of Denver for the age ranges indicated.

	17 TO 21 YEARS, INCLUSIVE			22 TO 26 YEARS, INCLUSIVE			17 TO 26 YEARS, INCLUSIVE		
	Cal./ hr./ sq. m	Cal./ hr / kg.	Cal./ hr / cm.	Cal./ hr./ sq. m.	Cal./ hr./ kg.	Cal./ hr./ cm.	Cal./ hr./ sq. m.	Cal./ hr./ kg.	Cal./ hr./ cm.
Mean	33.7	1.003	0.320	30.6	0.904	0.295	31.8	0.943	0.305
Standard deviation	1.6	0.107	0.019	1.4	0.072	0.018	2.1	0.100	0.022
Coefficient of variation	4.8	10.6	5.9	4.6	8.0	6.1	6.5	10.6	7.4

values decrease with increasing age, the means for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter are lower in the 22 to 26 year age group than in the 17 to 21 year period. The decrease in these values with increasing age will also explain why, except in two instances, the standard deviations and the coefficients of variation are lower for the 5 year period than for the 10 year period. When the values are considered from the standpoint of which method is the best for comparing the basal metabolism, it is evident that the coefficients of variation show a definitely lower dispersion for calories per hour per square meter than for calories per hour per kilogram and for calories per hour per centimeter. Accordingly, the first value, calories per hour per square meter, will be the only method used in the comparison with the literature that is to follow.

DISCUSSION

If altitude is a factor which raises the basal metabolism, as contended by McKittrick ('36) and by McCrery, Wolf and Bavousett ('40), the values for basal metabolism found in the present study should fall approximately midway between those obtained at Laramie and at Lubbock, which are about 2,000 feet higher and lower, respectively, than the elevation of Denver. However, that this is not the case may be seen from figure 1, which gives the mean values for the basal metabolism of the young women residents of Denver and those obtained elsewhere during the last 15 years in similar investigations on women between 17 and 26 years of age, inclusive. The mean of 31.8 calories per hour per square meter obtained at Denver is even lower than the 33.5 calories found by Mc-Crery, Wolf and Bavousett instead of being approximately halfway between this latter value and that of 35.6 calories found by McKittrick. Thus, the data herein presented do not support the thesis that altitude causes an increase in basal metabolism.

Obviously these divergent results concerning the effect of altitude on basal metabolism need clarification. The present

study supports the results of Lewis, Iliff, Duval and Kinsman ('43), who found no effect of change of altitude on the basal metabolism. Since both of these studies are in agreement with the literature on the subject, the question is raised as to whether McKittrick ('36), McCrery and Wolf ('38) and
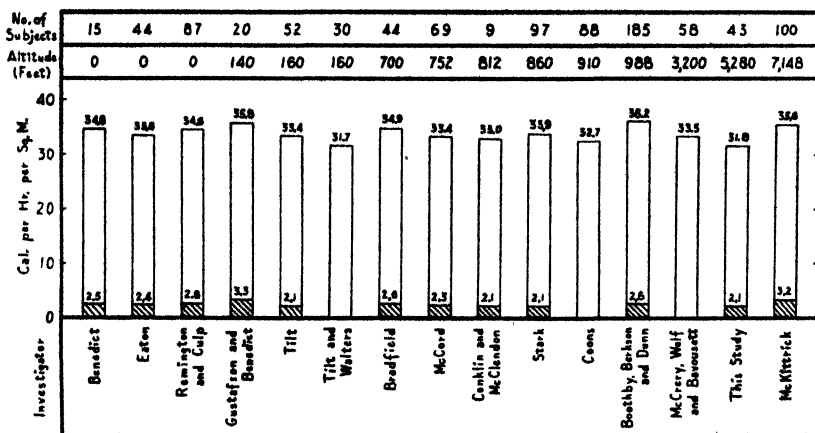
Fig. 1 Graphic comparison of the basal metabolism found in the present study and by other investigators for young women between 17 and 26 years of age, inclusive. The long rectangular column for each study shows the mean basal metabolism expressed as calories per hour per square meter. Where possible from the data given,[2] the standard deviation has been calculated for each study and is shown by the shaded area at the bottom of each column. The exact values for each mean and its standard deviation are given at the top of the respective columns which depict them. At the top of the graph above each column are to be found the number of subjects studied and the altitude at which each study was made.

[2] We are indebted to Dr. Juliana S. McCord for furnishing detailed records so that we could separate her data in the age range between 17 and 27 years of age from those of older women.

McCrery, Wolf and Bavousett ('40) are justified in drawing the conclusion that altitude affects the basal metabolism.

McKittrick ('36) compared the results of the determinations of basal metabolism which she obtained on young women residents of Laramie (altitude 7,148 feet) with those found by Tilt ('30) at Tallahassee, Florida (altitude 160 feet) and by Coons ('31) at Stillwater, Oklahoma (altitude 910 feet).

As may be seen from figure 1, the value reported by Mc-Kittrick is definitely higher than that obtained either by Tilt or by Coons. For this reason McKittrick concluded that the high value found in Laramie indicated that the high altitude at which these determinations were made raised the basal metabolism. Besides the literature to which McKittrick made reference, reports of several other investigators were then available for comparison. Tilt and Walters ('35) at Tallahassee (altitude 160 feet), Conklin and McClendon ('30) at Minneapolis (altitude 812 feet) and Stark ('32, '33) at Madison, Wisconsin (altitude 860 feet) agreed with Tilt and with Coons in that their mean values were definitely lower than that found by McKittrick. However, Gustafson and Benedict ('28) at Wellesley, Massachusetts (altitude 140 feet) and Boothby, Berkson and Dunn ('36) at Rochester, Minnesota (altitude 988 feet) obtained values very similar to that found by McKittrick. Furthermore, Benedict ('28) at Boston, Massachusetts (sea level), Remington and Culp ('31) at Charleston, South Carolina (sea level) and Bradfield ('27) at Columbia, Missouri (altitude 700 feet) reported that residents of these respective localities showed mean values for calories per hour per square meter which were only 1 calorie or less below that found by McKittrick. The lack of correlation between the basal metabolism found and the altitude at which the studies were made does not justify the conclusion that the relatively high basal heat production obtained on young women at Laramie is attributable to altitude.

McCrery and Wolf ('38) found the basal metabolism of young women at Lubbock (altitude 3,200 feet) when expressed as calories per hour per square meter to be lower than that reported by McKittrick ('36) at Laramie (altitude 7,148 feet) and higher than that obtained by Tilt ('30) at Tallahassee (altitude 160 feet). This work was expanded by McCrery, Wolf and Bavousett ('40), who compared the total calories found on young women residents of Lubbock with those obtained by Tilt, by Coons ('31) at Stillwater (altitude 910 feet), by Stark ('32, '33) at Madison (altitude 860 feet) and by

McKittrick. Since the values procured at Lubbock fell between those reported by Tilt, by Coons and by Stark, on the one hand, and those found by McKittrick, on the other, McCrery, Wolf and Bavousett concluded that their results indicated that altitude raises the basal metabolism. However, their procedure of comparing total calories does not take into account the size of the individual. When calories per hour per square meter are considered, the results of McCrery and co-workers do not show significant differences from those obtained by Tilt, by Coons and by Stark. Moreover, the mean value of 33.5 calories per hour per square meter obtained on young women of Lubbock is lower than those found by Benedict ('28) at Boston (sea level), by Remington and Culp ('31) at Charleston (sea level), by Gustafson and Benedict ('28) at Wellesley (altitude 140 feet), by Bradfield ('27) at Columbia (altitude 700 feet) and by Boothby, Berkson and Dunn ('36) at Rochester (altitude 988 feet); it is practically the same as those found by Eaton ('39) at New Orleans (sea level) and by McCord ('39) at Bloomington, Indiana (altitude 752 feet), and only 0.5 of a calorie higher than that found by Conklin and McClendon ('30) at Minneapolis (altitude 912 feet). Consequently, the conclusion of McCrery, Wolf and Bavousett that their results indicate that altitude raises the basal metabolism is unwarranted.

Consideration of the standard deviations for the basal metabolism reported by the different workers may throw some light on whether variation in the degree of dispersion of the data is a factor in the discrepancies observed. It will be seen from figure 1 that the values for standard deviation vary between 2.1 and 3.3 calories per hour per square meter and are within 0.5 of a calorie of one another, if the results of Gustafson and Benedict ('28) and McKittrick ('36) are excepted. The relatively high degree of dispersion of the data in these two studies may well explain why these investigators found higher values for calories per hour per square meter than any of the others cited with the exception of those of Boothby, Berkson and Dunn' ('36). Except for the two studies

noted, the degree of dispersion is very similar; hence, a comparison of the means should give an accurate picture of whether the basal metabolism is affected by altitude. A glance at figure 1 shows that no consistent relationship exists between the basal metabolism of the young women observed and the altitude at which the studies were made. This is even more evident, if one considers specific results. For example, the mean of 31.8 calories per hour per square meter obtained in the present study at Denver (altitude 5,280 feet) is similar to the 31.7 calories found by Tilt and Walters ('35) at Tallahassee (altitude 160 feet) and is lower than the values reported by the other investigators whose studies were made at elevations below 1,000 feet. Moreover, the value of 33.9 calories found by Stark ('32, '33) at an altitude of 860 feet is little different from the values obtained at a similar elevation by McCord ('39), at lower elevations by Eaton ('39) and by Tilt ('30) and at 3,200 feet by McCrery, Wolf and Bavousett ('40). Thus, a preponderance of evidence warrants the conclusion that, within the ranges of elevation cited herein, altitude does not affect the basal metabolism.

SUMMARY

1. Ninety determinations of basal metabolism on forty-three young women residents of Denver between the ages of 17 and 26 years, inclusive, are reported.

2. When the results of these determinations are compared with the values obtained in similar investigations at other altitudes during the last 15 years, the basal metabolism of the young women studied at Denver (altitude 5,280 feet) is found to agree closely with, or to be lower than, the observations on women of comparable ages at elevations below 1,000 feet.

3. No consistent relationship exists between the basal metabolism of the young women observed in all of these studies and the altitude at which the determinations were made.

4. The relatively high values for basal metabolism at the higher elevations that have been reported in the literature cited as due to altitude must be attributable to some other causative factor.

ADDENDUM

After this paper was accepted for publication, J. McCrery, M. Wolf Lamb and N. D. Bavousett published an article (J. Nutrition, vol. 25, p. 245, March, 1943) in which they state that the differences found in the basal metabolism of normal women of college age by various investigators "cannot be attributed to altitude." This conclusion is a complete reversal of the earlier opinion of these authors (McCrery, Wolf and Bavousett, '40) that the basal metabolism is affected by altitude.

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A STUDY OF THE AVAILABILITY OF THE IRON IN ENRICHED BREAD

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The addition of iron compounds either to flour or directly to dough mixes as a part of the bread enrichment program has caused a renewed interest in the availability of different forms of iron to the organism. Forms of iron insoluble in neutral solution, such as the pyrophosphate, have been used to a considerable extent for flour or bread enrichment, because of their convenience in handling and the negligible effects of such compounds on the color, flavor, and texture of the bread. In view of the wide extent of the enrichment program the comparative availability of the different types of iron compounds used for this purpose becomes a factor of importance. This matter has been investigated by studying the availability to anemic rats of two widely different iron compounds which are of interest for bread enrichment purposes; one of these is soluble, and the other insoluble.

Sodium iron pyrophosphate was used as the insoluble type of iron, ferrous sulfate as the soluble form.

The effect of ferric pyrophosphate in milk anemia of rats has been previously investigated by Elvehjem, Hart and Sherman ('33), but the results obtained by these authors are not comparable to those noted in this study, since the Wisconsin workers used a salt of ferric pyrophosphate together with citric acid which rendered it soluble (Elvehjem, '42). (The Merck Index ('40) describes a ''Ferric Pyrophosphate Soluble

N.F.VI." containing 12% Fe, 20% P_2O_5, and 40% citric acid, freely soluble in water.) This compound was found to be as available for hemoglobin regeneration as an equivalent amount of ferric chloride.

METHODS AND RESULTS

The anemic animals were prepared by rearing young rats on a ration of whole cow's milk, according to the procedure of Elvehjem and Kemmerer ('31). The animals were kept in rust-free galvanized iron cages, fitted with new wide mesh wire screens. No water was offered to the rats while on the milk ration, but those animals that were changed to a solid ration were supplied with water in glass bottles emptying into aluminum receptacles. The milk was fed in glass dishes.

In the first experiment, sodium iron pyrophosphate was compared with ferrous sulfate and ferric sulfate by administering the three compounds to groups of anemic rats which were maintained on a diet of whole milk throughout the test period. In the second experiment, after the production of milk anemia, the milk ration was discontinued and diets containing commercial iron-enriched bread were fed. The bread in one case contained sodium iron pyrophosphate, and in the other ferrous sulfate in an amount supplying exactly the same level of iron.

The sodium iron pyrophosphate [1] used was a double salt of the composition $Fe_4(P_2O_7)_3 \cdot 2 Na_4P_2O_7 \cdot 6 H_2O$. The actual iron content was found to be 15.99%, as compared to the theoretical value of 16.12%. The other salts used were Merck's Ferrous Sulfate Reagent ($FeSO_4 \cdot 7 H_2O$) and Merck's Ferric Sulfate Reagent ($Fe_2(SO_4)_3$ with about $6 H_2O$). The theoretical and observed iron content of the ferrous sulfate were 20.09% and 20.0%, for the ferric sulfate 21.99% and 23.8%, respectively.

Hemoglobin analyses were made by the acid hematin method, using a Klett-Summerson photoelectric colorimeter [2]

[1] Obtained from the Victor Chemical Works, Chicago, Ill.
[2] Made by the Klett Manufacturing Company, New York, N. Y.

for the measurement of the concentration of the solutions. The readings of this colorimeter were standardized with a series of acid hematin solutions made from a sample of blood of which the hemoglobin content was established by the oxygen capacity method of Lundsgaard and Möller ('22).

Iron analyses of the components of the diets were made by digestion in a mixture of nitric and perchloric acids, with development of color by α, α' dipyridyl as described in Cereal Chemistry Laboratory Methods ('41).

Activity of sodium iron pyrophosphate, ferrous sulfate, and ferric sulfate in anemic rats on a milk ration. The animals in this experiment were ready for testing at 7 weeks of age, with blood hemoglobin values of 2.8 to 4.5 (average 3.5) gm. per 100 cc. They were divided into groups of ten animals each and evenly distributed as to sex and average hemoglobin values. The pyrophosphate compound and the ferrous sulfate were given at two levels, supplying 0.15 or 0.30 mg. of iron per rat per day; the ferric sulfate was given only at the lower level. The ferrous and ferric sulfates were given in solution form; the sodium iron pyrophosphate was made into a 1% suspension with tragacanth. The solutions or suspension were administered into the animals' stomachs from a syringe by inserting a hypodermic needle tipped with a short fiber catheter. The preparations were given three times weekly in the amount of twice the "daily" dose. Some of the rats were rather feeble when placed on test, and a few died during the first week of the test period.

The average hemoglobin values for the various groups during the 4-week test period are shown in figure 1. It will be seen that the hemoglobin regeneration of 1.75 gm. in 4 weeks with 0.30 mg. of Fe as the pyrophosphate compound is considerably lower than the hemoglobin regeneration of 3.16 gm. noted in the series medicated with only 0.15 mg. of iron as ferrous sulfate.

These data indicate that sodium iron pyrophosphate is somewhat less than 50% as available for hemoglobin regeneration as ferrous sulfate under the experimental conditions used.

As would be expected, ferrous and ferric sulfates gave similar results. The ferric sulfate was tested in this experiment because of the still prevalent belief that clinically ferrous iron is much more effective than ferric iron salts. This finding of the similar availability of ferrous and ferric iron to anemic rats is in agreement with the report of Underwood ('38), and gives no support to the medical belief in the greater effectiveness of ferrous iron.
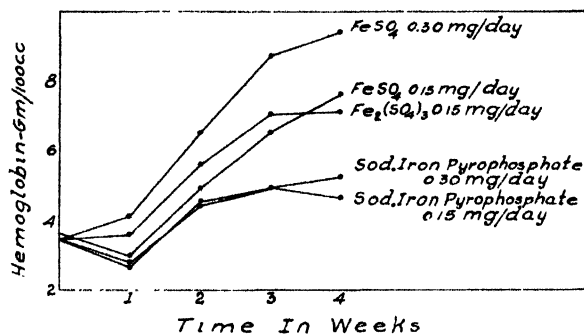
Fig. 1 Hemoglobin regeneration in anemic rats on a milk ration with different levels of ferrous sulfate, ferric sulfate, or sodium iron pyrophosphate. The figures at the right of each curve indicate the dosage levels in terms of milligrams of Fe per day. The curves are averages for the surviving seven to ten rats in each group.

Availability of the iron in enriched bread. Two lots of enriched bread, containing identical amounts of iron, were baked by a nationally known bakery. The iron contained in one lot of the finished product was in the form of sodium iron pyrophosphate, and in the other as ferrous sulfate.

Three diets were prepared from the dried and pulverized bread samples. All diets had the following percentage composition: dried bread 82, iron-low casein 12, cottonseed oil 3, and iron-free salt mixture 3. To each 100 gm. of the diet the following supplements were added: vitamin A concentrate 1500 I.U., viosterol 400 I.U., thiamine 400 µg., riboflavin 1200 µg., pyridoxine 400 µg., calcium pantothenate 4 mg., choline chloride 20 mg., inositol 20 mg., $CuSO_4 \cdot 5 H_2O$ equivalent to 2 mg. Cu, and $MnCl_2 \cdot 4 H_2O$, equivalent to 0.8 mg. Mn.

The iron-free salt mixture was prepared by modifying the U.S.P.XI ('36) salt mixture no. 2. The ferric citrate and sodium chloride were omitted and the sodium biphosphate replaced with potassium biphosphate, since bread contains adequate sodium chloride. The casein was included in the ration in order to insure a satisfactory protein composition, since the bread proteins are incomplete for satisfactory growth. It was found necessary to prepare this casein in the laboratory. Analysis of a sample of technical casein showed it to contain 0.045 to 0.190 mg. of iron per gram, irregularly distributed. A sample of commercial "vitamin-free" casein was found to contain similar amounts of iron. The laboratory-prepared casein had an iron content of 0.021 mg. per gram.

Diet 1 contained enriched bread prepared with sodium iron pyrophosphate; diet 2 contained bread prepared with ferrous sulfate; diet 3 contained an equal mixture of the above enriched breads with the addition of the iron supplement in the form of ferrous sulfate to the extent of 20 mg. of iron per 100 gm. of diet. Diet 3 was included for control purposes in order to prove that the basic ration would promote rapid hemoglobin production in the presence of optimum iron levels.

The iron contents of the enriched breads were found on analysis to be very similar, i.e., 0.0244 mg. per gram for the sodium iron pyrophosphate bread and 0.0249 mg. per gram for the ferrous sulfate bread. With the enriched bread constituting 82% of the ration and the casein (iron content 0.021 mg. per gram) 12% of the ration, it was calculated that 89% of the iron in the finished diet should be that furnished by the bread and 11% that furnished by the casein. Analysis of the finished diets indicated that the actual iron content agreed with the calculated content within 1%, showing freedom from contamination with iron from any other source.

Anemic rats were prepared as previously described and divided into three evenly-matched groups: fifteen rats each for diets 1 and 2; ten for diet 3. The average extent of hemoglobin regeneration on the different rations during the 4-week test period is indicated in figure 2. It will be seen that the

total 4-week gain in hemoglobin of 2.57 gm. per 100 cc. for diet 1 was somewhat less than half that of the 6.00 gm. gain for diet 2. This indicates that the iron in enriched bread containing iron as sodium iron pyrophosphate is less than 50% as available as that in enriched bread containing iron as ferrous sulfate. The very rapid increase of hemoglobin in group 3,
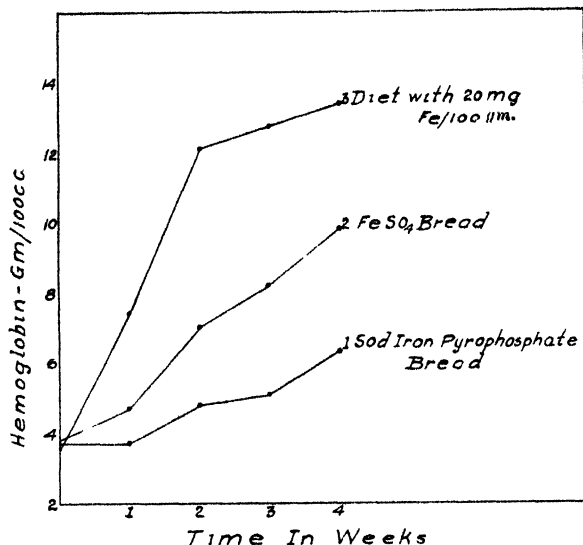
Fig. 2   Hemoglobin regeneration in anemic rats fed enriched bread. The curves are average figures for nine to fourteen rats per group.

fed a diet identical to that of groups 1 and 2, except that the iron content was ten times greater, indicates that the diet used will permit excellent hemoglobin regeneration in the presence of optimal iron.

It is, of course, necessary to know that the food consumption of the different groups of rats was similar, since the iron intake in this experiment was dependent upon the food intake. Food records kept during the first 3 weeks of the test period revealed that the average food consumption for groups 1, 2 and 3 was 11.0, 12.2 and 11.9 gm. per rat per day, respectively. The average growth rates for the three groups are shown in table 1.

TABLE 1

Growth rates of anemic rats fed enriched bread.

GROUP	NUMBER OF RATS COM- PLETING TEST	INITIAL WEIGHT	AVERAGE WEIGHT AFTER			
			1 week	2 weeks	3 weeks	4 weeks
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	14	66	87	108	128	137
2	11	65	94	115	137	145
3	9	64	93	115	138	146

It will be seen that both the food consumption and growth rates for groups 2 and 3 were practically identical, while the group 1 rats ate about 10% less food and grew a little more slowly. It is thus indicated that the much greater rate of hemoglobin regeneration in group 2 cannot be considered as due to the slightly greater food intakes.

Statistical evaluation of results. The data for the hemoglobin values at the end of the 4-week test period, both for the animals fed the enriched bread and those fed the iron salts, have been treated statistically in table 2.

TABLE 2

Availability of iron salts for hemoglobin production.

GROUP	MATERIAL FED	AMT FE PER DAY	NO OF RATS COM- PLETING TEST	AVERAGE HEMOGLOBIN VALUES		
				Initial	Final (after 4-week test period)	Standard deviation of final hemoglobin values
		mg		*gm /100 cc*	*gm /100 cc*	
			Experiment 1			
1	Sodium iron pyrophosphate	0.15	9	3.49	4.63	0.88
2	Sodium iron pyrophosphate	0.30	10	3.45	5.20	0.96
3	Ferrous sulfate	0.15	8	3.60	7.60	0.92
4	Ferrous sulfate	0.30	7	3.53	9.44	1.38
5	Ferric sulfate	0.15	8	3.51	7.06	1.82
			Experiment 2			
1	Sodium iron pyrophosphate bread		14	3.68	6.25	1.05
2	Ferrous sulfate bread		11	3.78	9.78	1.40
3	Diet with 20 mg. Fe per 100 gm.		9	3.53	13.36	1.24

In examining the results for significance, we may compare the final average hemoglobin value of group 1, fed sodium iron pyrophosphate in the amount of 0.15 mg. Fe per day, with that of group 3, fed ferrous sulfate also at the level of 0.15 mg. Fe per day. Dividing the difference of 2.97 in mean hemoglobin values by the standard deviation of 0.92, we get the value 3.23. Since the difference between groups is ordinarily considered significant when the mean difference is at least twice the magnitude of the standard deviation, the results may be considered indicative of a real significance. Comparing group 2, given 0.30 mg. Fe per day as sodium iron pyrophosphate with group 4, given 0.30 mg. Fe per day as ferrous sulfate, the mean difference in final hemoglobin values is 4.24. Dividing by 1.38, the standard deviation (S. D.) of the most variable group, we get 3.07 which may be taken as indicating a significant difference. There is also a significantly greater final hemoglobin value for group 3, receiving 0.15 mg. Fe as ferrous sulfate than for group 2, receiving 0.30 mg. Fe as sodium iron pyrophosphate, the difference in mean values being 2.50 S. D.

In experiment 2, the significance of the results is indicated by the fact that the mean final hemoglobin value for the group given bread baked from flour containing ferrous sulfate is 2.52 S. D. greater than that of the group given bread containing sodium iron pyrophosphate.

SUMMARY

Sodium iron pyrophosphate, administered to rats maintained on a diet of whole milk, has been found to have an availability of considerably less than 50% as compared to the utilization of ferrous or ferric sulfate. Likewise, when enriched bread containing sodium iron pyrophosphate or ferrous sulfate was fed to anemic rats, the availability of the former compound was less than 50% of that of the ferrous sulfate.

After this paper was submitted, an article appeared by Nakamura and Mitchell ('43), which calls for comment. These workers reported that the utilization of iron as sodium iron pyrophosphate or ferric chloride is equally satisfactory as tested in anemic rats. It is difficult to reconcile these findings with those reported here. Mitchell ('43) has pointed out that in their experiments the iron compounds were administered daily, in contrast to the thrice-weekly administration of the iron salts in the present study. However, while this might account in part for the difference observed when the iron salts were administered as such, it would not explain the poor utilization of sodium iron pyrophosphate when fed in enriched bread ad libitum. It should also be pointed out that while the preparations of sodium iron pyrophosphate were obtained from the same manufacturer, they were, of course, different samples, and this might to some extent account for variation in degree of utilization. No doubt further work will clarify this matter.

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PREVENTION OF PEROSIS AND DERMATITIS IN TURKEY POULTS [1]

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It is recognized that manganese is necessary for the prevention of perosis in chicks (Wilgus, Norris and Heuser, '37); however, Jukes ('40) found both manganese and choline essential for protection of poults from perosis. Jukes ('40), Hogan et al. ('41) and Record and Bethke ('42) established the essential rôle of choline in the prevention of perosis in chicks.

It was recently reported that in addition to choline, there exists at least two other organic nutrients concerned in the prevention of perosis in chicks. One of these substances is biotin (Jukes and Bird, '42), and the other substance (Richardson and Hogan, '41), not identical with any of the recognized vitamins, is present in the aqueous extract of desiccated beef liver. This unrecognized substance was adsorbed on fuller's earth and subsequently eluted with ammonium hydroxide. Perosis has been reported to accompany the egg white injury syndrome in chicks (McElroy and Jukes, '40), which suggests that this symptom is a result of induced biotin deficiency.

Hegsted et al. ('40, '42) and Ansbacher and Landy ('41) established the essential rôle of biotin in the prevention of a scaly dermatitis in chicks which was distinct from the dermatitis produced by pantothenic acid deficiency. Lepkovsky and Jukes ('36) and Jukes ('38) reported that vitamin G was an

'anti-dermatitis' vitamin in poult nutrition. Patrick, Boucher, Dutcher and Knandel ('41) reported that poults receiving a simplified ration containing a yeast residue or crystalline biotin methyl ester did not develop dermatitis even when the ration was grossly inadequate in riboflavin. Supplementation with 8 μg. of biotin daily gave complete protection. Perosis was frequently observed in this laboratory in turkey poults which received rations adequate in choline and manganese. Dried brewers' yeast was found to be effective in preventing perosis under these conditions. This paper reports studies of the anti-perosis properties of yeast fractions, crystalline biotin methyl ester and choline. Further evidence on the effectiveness of biotin and ineffectiveness of riboflavin in the prevention of dermatitis is also presented.

EXPERIMENTAL

The composition of the simplified basal ration is given in table 1. Bronze and White Holland turkey poults were used throughout the experiment. They were started when 1 day old and kept in electrically heated brooders equipped with

TABLE 1

Composition of the simplified basal ration.

	%
Purified casein	25
Extracted wheat bran	20
Salt mixture no. 30 [1]	4
Manganese sulfate	0.04
Soybean oil [2]	5
Cod liver oil (400 D)	2
Wheat germ oil [3]	2
Polished rice	5
Choline	0.2
Pyridoxine	0.0005
Thiamine	0.0005
Vitamin carriers as described in tables 2 and 3	
Corn starch to make 100%	

[1] Hubbell, Mendel and Wakeman ('37).
[2] Kindly furnished by Central Soya Co., Fort Wayne, Indiana.
[3] Kindly furnished by General Mills Inc., Minneapolis, Minnesota.

hardware cloth floors. The experimental ration and water were fed ad libitum.

The vitamin carriers were prepared from strain S dried brewers' yeast [2] as follows: (1) The yeast was extracted five times with 95% ethyl alcohol at 65°C. (2) The alcohol insoluble fraction was extracted ten times with boiling HCl-acidulated water at pH 4. (3) The residue was not treated further. (4) The acidulated aqueous extract from 2 was concentrated in vacuo and fed as such or fractionated in the following manner: (A) Alcohol-precipitate and filtrate-fraction: these fractions were prepared by adding enough 95% ethyl alcohol to obtain maximum precipitation and then separated by decantation. (B) Norite and fuller's earth eluates: these fractions were prepared by treating the acidulated aqueous extract, after being concentrated to a convenient volume, with norite or fuller's earth and eluting the adsorbates with 0.2% ammonium hydroxide, thus forming the norite or fuller's earth eluate.

The extracted wheat bran was prepared as follows: (1) Washed once with tap water at 60°C. (2) Washed five times with 0.1% ammonium hydroxide at 60°C. (3) Washed eight times at 60°C. with HCl-acidulated water at pH 4. (4) Washed with cold tap water until free of acid and then dried in a forced draft oven at 80°C.

Biotin concentrate I was prepared from liver residue according to the method of du Vigneaud et al. ('41), and biotin concentrate II (S.M.A.-200) and crystalline biotin methyl ester were purchased from a commercial concern.[3]

The influence of riboflavin on dermatitis and perosis

A summary of results obtained by feeding varying levels of riboflavin is presented in table 2.

Basal ration 1 had the following precentage composition: simplified basal 92 (table1), alcohol precipitate of yeast fraction 3, feeding cane molasses 5, and nicotinic acid 0.001. The

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[2] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.
[3] S.M.A. Corporation.

alcohol precipitate of yeast fraction was added as a source
of unrecognized vitamins in which the simplified basal ration
was likely to be deficient. The feeding cane molasses was
added as a source of pantothenic acid. The complete ration
was assayed for riboflavin in two laboratories by the fluoro-
metric method: the laboratory of the Cooperative G. L. F. Ex-
change, Inc., Buffalo, N. Y. reported 155 μg. per 100 gm.; 160
μg. per 100 gm. was found in this laboratory.

TABLE 2

Effect of graded levels of riboflavin on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 1 [1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMA-TITIS	PEROSIS
		%	%
Riboflavin			
None	16	87	25
200 μg.....	12	92	50
230 μg.	17	76	53
260 μg.	16	75	56
290 μg.	18	72	33
320 μg.	18	67	28
350 μg.	15	67	67
380 μg.	11	45	36
380 μg. plus 10% dried brewers' yeast	15	none	none

[1] Riboflavin expressed as micrograms per 100 gm. of ration.

Severe dermatitis and perosis occurred frequently by the
end of the third week in the poults of all groups receiving
varying levels of riboflavin, while a control group of poults
receiving 10% dried brewers' yeast developed normally. The
dermatitis involved the feet, anus, mouth, eyes, and most
proximal portion of the upper beak. The accumulation of
exudate and the severity of the dermatitis around the eyes
and mouth may have been aggravated by a secondary infection.
In many instances, when the poults lived long enough, the
surface of the skin appeared scaly and felt like parchment and
the wings became rigid, due to dermatitis involving the skin
of this area, while hemorrhagic fissures appeared in the der-
mis of the feet. The quantity of riboflavin in the ration did

not appear to influence the time of onset or incidence of either dermatitis or perosis. The type of perosis present was dissimilar to that observed in poults receiving rations low in choline or manganese. The legs appeared weak and although the tendon of Achilles seldom slipped permanently from its condyle, the outward manifestation was a wobbly gait and a loose-joint appearance in the hock joints. The poults stood with their hocks further apart than normal and the angle formed by the tibia and metatarsus was increased until a 'peg-leg' appearance resulted.

The use of choline, biotin, and yeast fractions in the study of dermatitis and perosis

Following the experiments which showed that riboflavin did not influence the incidence of dermatitis, the work was extended to include the rôle of biotin, choline and various fractions of yeast in turkey nutrition. These results as related to dermatitis and perosis are recorded in table 3.

Poults receiving rations adequate in biotin but inadequate in any fraction of the aqueous extract of yeast or in riboflavin were protected from dermatitis but not from perosis. The alcohol-precipitate fraction when fed as the only vitamin concentrate added to the basal ration was ineffective in the prevention of perosis or dermatitis. A combination consisting of a biotin concentrate with either the ammonium hydroxide eluate or the alcohol-precipitate fraction was required to protect the poults from perosis. A ration containing 30 parts of untreated fresh egg white and 70 parts of a practical ration produced perosis and dermatitis in poults. The incidence of perosis did not always parallel the incidence of dermatitis in poults receiving rations deficient in biotin. This agrees with the report of Jukes and Bird ('42) that with chicks a smaller intake of biotin is required to prevent perosis than is required to prevent dermatitis. The above authors reported that 0.34 µg. of biotin per chick per day was enough to completely protect the chick from perosis but not enough for protection against dermatitis. We were unable to show that inositol either increased

or decreased the incidence of perosis; however, choline, when fed at a level of 0.2% in addition to 10% strain G dried brewers' yeast [4], gave complete protection from perosis. A

TABLE 3

Effect of choline, biotin and yeast fractions on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 2 [1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMA-TITIS	PEROSIS
		%	%
Strain G yeast			
Without choline	18	0	78
Plus 0.05% choline	12	0	33
Plus 0.10% choline	10	0	30
Plus 0.15% choline	9	0	11
Plus 0.20% choline	17	0	0
Basal only	8	100	50
Yeast residue	30	0	53
Without riboflavin	22	0	45
Plus aqueous extract of yeast	26	0	0
Plus norite eluate	18	0	0
Plus norite eluate plus inositol	17	0	0
Plus fuller's earth eluate	10	0	0
Plus inositol	16	0	56
Plus 1% salt mixture no. 30	17	0	47
Aqueous extract of yeast	10	60	0
Biotin II	6	0	50
Alcohol precipitate	31	84	71
Plus biotin I	10	0	0
Plus biotin II	10	0	0
Plus crystalline biotin methyl ester [2]	5	0	0
Aqueous extract of yeast			
Plus biotin I	11	0	0
Plus biotin II	12	0	0
Practical ration	10	0	0
Plus 30% raw egg white	10	70	60

[1] Basal ration 2 was composed of the simplified basal ration (table 1) plus 200 μg. Ca-pantothenate, 500 μg. riboflavin and 200 mg. choline per 100 gm. of ration, unless modified as indicated in the body of the table.

[2] Crystalline biotin methyl ester was fed by pipette at a level of 2.5 μg. on the 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, and 21 days of age.

[4] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.

combination of choline, biotin and a factor or factors which can be adsorbed on norite or fuller's earth and eluted with ammonium hydroxide appear to be required for prevention of perosis in turkey poults.

SUMMARY

1. Riboflavin did not influence the incidence of dermatitis in turkey poults.

2. Biotin was found to be an anti-perosis and an anti-dermatitis factor.

3. The anti-perosis property of choline was confirmed.

4. Inositol did not influence the incidence of perosis.

5. One or more factors present in an eluate from an adsorbate of an aqueous extract of dried brewers' yeast possessed anti-perosis properties.

6. It appears, therefore, that at least three organic factors are required to protect the poult from perosis, namely, choline, biotin and an unrecognized factor or factors which can be adsorbed on fuller's earth or norite and subsequently eluted with ammonium hydroxide.

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STUDIES ON THE NUTRITIONAL REQUIREMENTS
OF THE RHESUS MONKEY [1]

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A large part of our present knowledge of nutrition has been
obtained through the use of small experimental animals.
Early studies with rats, mice, chicks, and guinea pigs made
it possible to differentiate between the fat soluble and the
various water soluble vitamins. Dogs, calves, and pigs have
been used in the more recent investigations and work with
these animals has aided in estimating the human requirements
for the various food factors. Experiments on induced vitamin
deficiencies in humans have done much to clarify the clinical
picture of frank avitaminosis as well as the subclinical type of
nutritional deficiency, but such studies are obviously limited.
It would seem probable that additional knowledge of the
nutrition of man could be obtained through the use of a more
closely related animal, namely, the monkey.

The literature dealing with nutritional experiments in which
monkeys have been used is not extensive. Early experiments
dealt with the production of a pernicious anemia-like syndrome
in monkeys (Wills and Bilimoria, '32) by feeding a diet now
known to be deficient in several factors. More recent experi-
ments by this same group of workers (Wills, Clutterbuck and

Evans, '37; Wills and Stewart, '35) have shown that some factor as yet unidentified is concerned in the production and cure of certain anemias, primarily of the pernicious type. The most complete work within the past several years has been that of Day and his collaborators. In a series of papers (Day et al., '35; Day et al., '38; Langston et al., '38; Day et al., '40)` evidence has been presented that monkeys fed a modification of the classical blacktongue producing diet of Goldberger (Goldberger et al., '28) fail to survive and are incapable of maintaining a normal blood picture, the distinguishing feature being the abnormally low white cell count. These investigators postulate that some factor in yeast or in liver, which they call "vitamin M," is required by these animals for maintaining normal growth, mouth structure and blood elements.

Other work on the nutrition of the monkey has been reported by Topping and Fraser ('39) who described the mouth lesions associated with dietary deficiencies in this animal. Tomlinson ('39) has presented the symptomatology and histopathology of the mouth lesions in acute vitamin A, vitamin D, nicotinic acid and riboflavin deficiencies. Fraser ('42) has described the chronic deficiency of calcium, vitamin C and a combination of the two deficiencies in monkeys, and Fraser and Topping ('42) have described mouth lesions associated with these deficiencies. Experimental beriberi and spontaneous polyneuritis have been observed in Macaca sylvanus by Leblond and Chaulin-Serviniere ('42). Although they were not primarily concerned with nutritional studies, Johnstone and Reed ('37) attempted to produce a "vitamin G" deficiency. Harris ('37) differentiated the antiblacktongue and the P–P factors from lactoflavin and vitamin B_6 through the use of monkeys. Several older papers dealing with dietary studies in monkeys have appeared in the literature but many of these have been reviewed in the literature cited. Monkeys have been used for experiments designed to study some phase of physiology, bacteriology, immunology, or pharmacology, but in many cases the workers have neglected to consider the effect of nutrition on their experiments.

One phase of work in this laboratory deals with the role of nutrition in the susceptibility to poliomyelitis and perhaps other infectious diseases, but before setting out on a comprehensive study of the importance of nutritional resistance to infection, it was necessary to determine the normal nutritional requirements of monkeys. No complete study has been reported on the use of the "synthetic diet" in the feeding of monkeys. Previous investigations in this laboratory (Henderson, McIntire, Waisman and Elvehjem, '42) have shown that rats are able to grow and reproduce on such so-called "synthetic diets." Extensive work has also shown that guinea pigs (Sober, Mannering, Cannon, Elvehjem and Hart, '42) and chicks are unable to survive on diets similar to the one to be described below.

EXPERIMENTAL

Each monkey was kept in a roomy individual cage, equipped with both a small sliding door and a larger front door, a screen floor and a removable litter tray. The feed and water were furnished in 1 pound glazed porcelain butter jars suspended in holders under the smaller door, thus keeping the food off the floor and avoiding breakage of the feed cups and excessive waste of the food. All cages and other equipment used in these experiments were kept clean by frequent washing and steaming. The animals were trained to go into a tared weighing box and were thus weighed to within 5 gm. whenever it was deemed desirable to secure body weight data. Usually the monkeys were weighed twice weekly, or more often as indicated by the experimental procedure, specifically during nutritional failure or remission of symptoms because of the therapy being administered.

The diet (M-3) used throughout these studies consisted of sucrose 73 parts, purified vitamin-free casein[2] 18, salt mixture[3] 4, corn oil 3, cod liver oil 2. The vitamin supplements

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[2] Both Smaco-vitamin free casein (SMA Corp.) and Labco-vitamin free casein (Borden Company) were used with equal success.

[3] Phillips, P. H., and E. B. Hart. J. Biol. Chem., vol. 109, p. 657, 1935.

were given to each monkey in the water dish at the day's first feeding in a minimum amount of water, slightly sweetened to improve palatability. The daily vitamin supplements contained the following: thiamine hydrochloride 1 mg., nicotinic acid 5 mg., riboflavin 1 mg., pyridoxine hydrochloride 1 mg., calcium pantothenate 3 mg., choline chloride 50 mg., para-aminobenzoic acid 100 mg., i-inositol 100 mg., and vitamin C 25 mg. The individual vitamin solutions were made up at least once a week, and kept in brown bottles in concentrations that were convenient for easy measurement, so that a particular vitamin could be omitted from the daily supplement when desired. The monkeys usually drank the daily vitamin supplements at once. In no case was water furnished to the animals until the daily supplements were consumed. Adequate quantities of the particular diet were given twice daily; each feeding consisted of 100–200 gm., depending on the size of the monkey. When a diet was altered, the inclusion of the particular addition to the diet was made at the expense of the 95 parts of the mixture of dry ingredients. If the monkeys were given a liver fraction as therapy in an attempt to obtain remission of the loss in weight and specific symptoms, the solution of the liver was given with the vitamin supplements. If the animal did not consume it voluntarily, it was administered by stomach tube, no difficulty being encountered in giving as much as 50 cc. by tube daily. Tube feeding was discontinued as soon as the animal indicated a desire to drink the supplement.

The Rhesus monkeys used in these experiments were obtained through animal dealers and were tested immediately upon arrival with old tuberculin (OT) and with a purified protein derivative (PPD), one preparation in each eyelid subcutaneously. Chest plates were taken of individual monkeys in several groups in addition to the tuberculin tests, but in more recent shipments we have been satisfied with the results of the tuberculin tests only. The animals were retested with the tuberculin preparations at approximately 3–4 month intervals so that the hazard from this disease as well as the loss

to the experiments could be reduced to a minimum. Out of a total of one hundred monkeys on experiment three animals were discarded when the routine checkup indicated that they had the disease, and only three others have come to autopsy showing signs of tuberculosis.

Since the experiments were planned to study growth responses of the animals it was necessary to obtain small healthy monkeys. In no case were monkeys placed on experiment if they weighed as much as 3 kg. or if they were over 2–3 years of age as indicated by observation of their dentition. The starting weights of monkeys ranged from 1 kg. to 2.5 kg., and the age from about 1 to 3 years. Blood examinations were made at frequent intervals throughout the experimental periods. Hemoglobin was determined in the Evelyn photoelectric colorimeter and red and white cell counts were made in the usual manner from blood drawn from the marginal vein of the ear. Stool cultures were taken during the course of the experiment in order to check the presence of intercurrent intestinal infections.

The first experiments were planned so that growth responses in monkeys fed the synthetic diet could be compared with those obtained in animals fed the synthetic diet with a variety of liver fractions. Of the more than one hundred monkeys used in our studies thirteen have been allowed to remain on the basal diet plus the vitamin supplements until death. Of these thirteen, one was in very poor condition when received, and one had tuberculosis. The remaining eleven animals gradually lost weight and died within 28 to 119 days. All eleven animals appeared cachectic, and showed a definite gingivitis with unnatural stains on the teeth, an unkempt blackish appearance of the fur, occasional transient edema of the face, and anorexia. At autopsy each animal showed extensive gingivitis with occasional necrosis of the gums, often involving the mucous lining of the cheek. Four monkeys were able to survive long enough to develop extensive, gangrenous, perforating, necrotic areas of the face which began as infectious foci on the inner cheek. Gross examination of the heart, spleen, kid-

neys, pancreas, adrenals, sex glands and muscle showed no abnormal appearance. The lungs were normal in nearly all cases except for an occasional calcified tubercle or signs of hypostasis usually seen in the highly cachectic animals. The gastrointestinal tract showed petechial hemorrhages throughout, with less involvement of the stomach than the intestines. Tissues from all animals were taken for microscopic examination.

TABLE 1

Growth and blood data of monkeys receiving synthetic diet and of those receiving various liver fractions.

SYNTHETIC DIET PLUS ALL 9 VITAMINS PLUS	NUMBER OF MONKEYS AND SEX	AVERAGE GRAM GAIN PER DAY	RANGE OF GAIN PER DAY IN GRAMS	AVERAGE BLOOD PICTURE		
				Red blood cells per c mm	White blood cells per c. mm	Hemoglobin per 100 ml
				m llions	*thousand*	*grams*
(No supplements)	7 ♂		.83–13.9	4.33	9.66	12.3
	4 ♀		1.76– 8.15	4.40	8.17	12.7
3% whole liver substance	1 ♂	8.45		5.17	18.92	14.4
	1 ♀	6.5		4.53	14.32	13.7
1% liver extract	1 ♂	21.5		4.98	16.21	12.7
	1 ♀	7.65		5.94	19.20	12.6
3% liver extract	7 ♂	5.1	3.3 – 7.9	5.61	19.59	12.8
	5 ♀	7.3	4.2 –14.8	5.54	18.44	12.6
1% solubilized liver residue	2 ♀	7.5	7.0 – 7.9	5.51	12 98	12.5
3% solubilized liver residue	2 ♂	5.6	4.0 – 7.2	5.17	18.55	13.4
	2 ♀	3.1	3.0 – 3.2	5.18	17.09	12.4

The blood picture of monkeys kept on the basal diet indicated that during the onset of the deficiency and at death there was a slight reduction in the number of red blood cells, and a definite drop in the number of white blood cells. Moderate degrees of anemia were observed in those animals receiving the basal deficient diet since the hemoglobin values during the plateau in weight and during acute deficiency were somewhat lower than in those getting whole liver or liver extract. The figures for hemoglobin in table 1 are the averages for the

entire period. The white cell count averaged for the monkeys listed in the table reflects the count during the onset of the deficiency whereas in some monkeys the count actually dropped from 5000 to 2000 the day before death. In one monkey, the white count rose very abruptly prior to death and autopsy showed consolidation of the lungs and the presence of adhesions in the thorax. It seems probable that the white cell count rose in response to the infection after having gone down due to the deficiency. Leucopenia was a consistent finding in the deficient animals and had no bearing on the slight anemia.

All the monkeys fed the basal diet exhibited loose, mucous stools during the period of weight loss and the stool specimens showed positive cultures of Shigella paradysenteriae.

Twenty-two monkeys were given diets containing varying amounts of different liver fractions and table 1 summarizes a portion of the data on these animals. All the animals on the liver diets showed good growth, and are continuing in excellent health and showing characteristic sexual changes. Since many factors influence the response of the individual animal to a particular diet, such as duration of experiment, previous nutritional history, genetic variability, age and weight at start of the experiment, no attempt is made to appraise the relative value of the particular liver fraction, and the table lists only the range of the gain per day to show the variability in growth responses. It is obviously an error to insist that an average of the responses of several monkeys is a true indication of the merit of the liver fraction. The curves shown in figure 1 give typical responses of individual monkeys on the basal diet together with those of animals given whole liver, a water soluble portion of liver (liver extract) and an enzymically hydrolyzed fraction remaining from the water extract of the liver (solubilized liver residue). Several of the monkeys have been kept on the diet plus 3% liver extract for more than 575 days with continued gain in weight. Individual growth records also furnish evidence that whole liver is as effective as liver extract in supporting growth at a 3% level. The solubilized liver residue appears to give a slower rate of

growth than other fractions although the monkey appears to
be in a good state of nutrition. Some monkeys kept on solu-
bilized liver for long periods show some definite change in
the fur and skin.
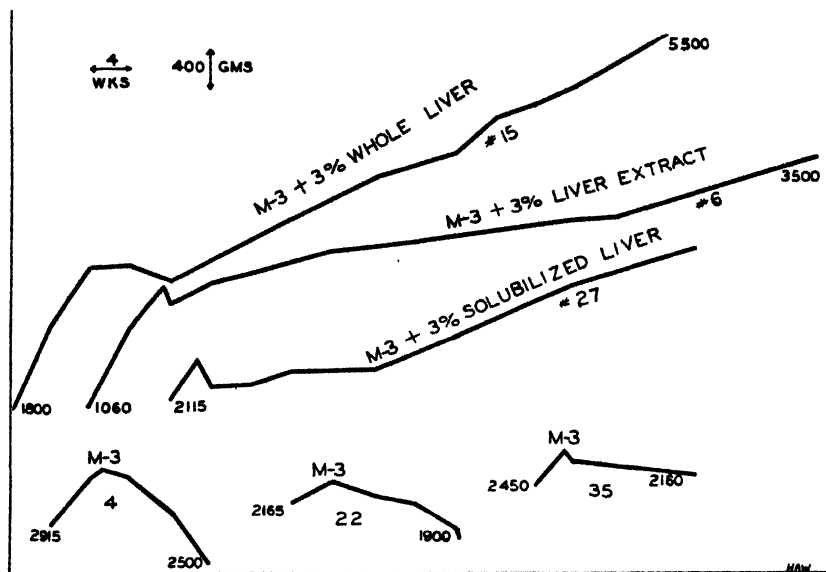
Fig. 1   Typical responses of monkeys on basal synthetic diet and on various
liver products.

DISCUSSION

It is evident from these data that monkeys are unable to
survive upon a "synthetic diet" containing sucrose, purified
casein, mineral salts, corn and cod liver oils, and the known
crystalline and readily available members of the vitamin B
group, thiamine, riboflavin, pyridoxine, nicotinic acid, choline,
calcium pantothenate, inositol, and para-aminobenzoic acid to-
gether with vitamin C. The gain in weight in the monkeys
fed this diet is only transitory and once the stores in the body
are depleted, there is a leveling off of the weight curve fol-
lowed by a gradual failure with increasing cachexia and final
death. The onset of secondary infections, the anemia, leuco-
penia, and gingivitis are outward indications of upset metabo-

lism and lowering of the natural defenses of the body. The moribund condition is accompanied by anorexia and a cyanotic appearance of the skin, giving the black dull-appearing fur. There is a marked reduction in the normal reflexes of the animal with an accompanying loss of activity. This condition is quickly improved by the feeding of any of the three liver preparations, whole liver, liver extract or solubilized liver residue.

During the course of these experiments it became evident that if a monkey kept on the basal diet was to be saved from certain death, it was necessary to administer one of the liver preparations before too sharp a loss in weight was noted. This procedure was resorted to in more than twenty monkeys, and in several of the animals the curative action of liver was demonstrated two or three consecutive times. In the curative trials, as well as in the prophylactic experiments, 3–5 gm. of any of the three liver fractions administered daily caused remission of symptoms within a period varying from 4 to 21 days. Figure 2 illustrates a typical response of a monkey to liver extract therapy after showing nutritional failure on the basal diet. It should be noted that feeding 20 µg. biotin [4] per day for 60 days during the period of nutritional failure had no effect on the course of the deficiency. The immediate growth response to a short period of liver extract feeding (5 days) is followed by a tendency to drop sharply after a few weeks. Liver therapy again at this point is followed by a sharp gain in weight which persists for a longer period before the plateau occurs, since the liver was given for 14 days. The blood picture is also charted and demonstrates the slight anemia and accompanying lowering of red blood cells during the more severe deficiency state. Since no anemia was present when the weight dropped the second time, the deficiency was apparently not as acute as the previous time. The white cell counts at appropriate intervals are also given in the figure, showing the leucopenia that exists during deficiency and its remission after therapy. The white cells are apparently more sensitive to

acute deficiency and to therapy as reflected in the respective low and normal counts.

The length of time elapsing between the start of the experiment and the death of monkeys fed the basal diet has varied between rather narrow limits when one considers the number of factors which can influence the results obtained. The average survival time of the eleven monkeys on the basal diet was 88 days; the range was 28 to 119 days. Only three monkeys
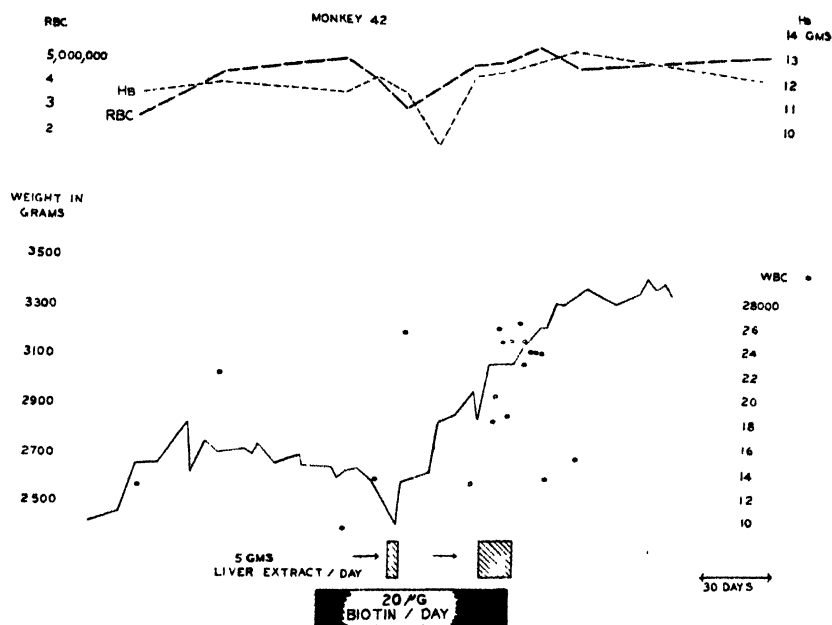
Fig. 2   Typical response of a monkey to liver extract therapy.

survived less than 88 days, the intervals being 28, 53 and 56 days. Although the weight and approximate age of the animal was known at the start of the experiment, the maximum gain in weight differed in the monkeys so that, whereas one animal gained 75 gm. in 91 days before death, another gained 665 gm. in a similar period of 87 days. It should be pointed out that each monkey must be considered individually with a recognition of the genetic factors which may influence the experi-

ments. Throughout the experiments, before each monkey received a particular fraction of liver which was prepared either from liver extract or from solubilized liver residue, the animal received the basal diet until the first drop in weight. In this way each animal served as its own control and one could judge the growth response with more reliability.

The first twenty-five animals which were fed the various diets were given the six well-established crystalline members of the vitamin B group in addition to vitamin C. Later animals received in addition to these i-inositol and para-aminobenzoic acid (PABA). We could observe no difference in the growth of those animals receiving these added vitamins and those which were given the first seven members. Again, no differences were noticeable in those animals fed the various percentages of liver with or without inositol and PABA.

The symptoms observed in our animals during the height of the deficiency may be the result of a single deficiency, but this has as yet not been established. The deficiency may actually be multiple in character and the symptoms observed could be manifestations of a variety of etiological factors.

It is evident that at least one, but more likely several factors required by the monkey are present in the liver. There appear to be no great differences between whole liver and liver extract since the animals grow equally well on both these products. The evidence is accumulating that the solubilized liver residue is not qualtitatively equal to the other liver preparations since a definite change occurs in the animals kept on this diet for a long period. Investigations on this point are being pursued.

From our observations on the animals kept on the basal diet and upon the same animals which have been autopsied, we must conclude that there is an impairment of the natural barriers against infection in these animals. The autopsies have furnished confirmation of this observation since an inflammatory gingivitis of the gum is seen at the teeth margins, and when the infection spreads to adjacent surfaces of the inner cheek ulceration of the tissues results. The various liver preparations are able to prevent or alleviate this condition.

During nutritional failure of several monkeys, the local infection was cured by administration (1 gm./day) of sulfathiazole in the form of the sodium salt. Although the infection cleared, nutritional failure persisted but was delayed somewhat when the invasion processes of the infection were eliminated. An interesting observation was made in the treatment of one deficient monkey given liver extract by stomach tube over a long period. The extensive ulcerated cheek (noma-like condition) was a complicating factor in the nutritional recovery of this monkey. After several 2 gm. daily feedings of sodium sulfathiazole the beneficial effect of the liver extract was visible in that growth was resumed, and even though the sulfa drug was discontinued and the ulcer still purulent, the liver extract apparently aided a return of the natural defenses with the resulting elimination of the infection and the beginning of the repair processes which ultimately completely filled in the cheek.

Further evidence of lowered resistance to infection in nutritional failure was obtained from gross observations of the gastrointestinal tract of monkeys fed the basal diet. Petechial hemorrhages were commonly observed in the mucosa of the entire intestinal tract in deficient animals, together with necrosis and ulceration of the colon in monkeys with frank bacillary dysentery. The presence of dysentery in vitamin deficient animals has been described by other workers (Janota and Dack, '39; Verder and Petran, '37; Topping and Fraser, '39; and Day et al., '40). Cultures of the stools of monkeys during the deficiency state tend to confirm previous reports.

SUMMARY

1. Monkeys are unable to survive upon a purified diet containing sucrose, casein, salts, corn oil, and all eight readily available members of the B group of vitamins and vitamin C.

2. The deficient monkeys show a loss in weight, anorexia, leucopenia, slight anemia, cachexia, and intercurrent infections, especially bacillary dysentery.

3. Any one of three liver products, whole liver, liver extract, and solubilized liver residue when fed at a 3% level in the diet, supports good growth, maintains the normal blood picture and tends to prevent dysentery and other secondary infections.

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DIGESTIBILITY OF CERTAIN HIGHER SATURATED FATTY ACIDS AND TRIGLYCERIDES

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INTRODUCTION

In view of the rather wide differences which the writers ('40) have found between the digestive coefficients of certain fats, it has seemed desirable to determine the digestibility of several pure saturated fatty acids and the corresponding triglycerides. Digestion tests have been conducted with mature male rats with diets containing different percentages of pure stearic, palmitic, myristic, and lauric acids and with pure tristearin, triplamitin, trimyristin, and trilaurin.

PREVIOUS INVESTIGATIONS

In experiments with dogs, Arnschink (1890) found tristearin to be 9–14% digestible. Nearly all of the undigested stearin was present in the feces as the triglyceride. Lyman ('17) obtained digestive coefficients of approximately 95% for glyceryl palmitate and 82% for palmitic acid. He states that the melting point of an ester is not the only, and probably not the chief, factor determining the rate of hydrolysis and absorption.

MATERIAL AND METHODS

Stearic, palmitic, myristic, and lauric acids and the corresponding triglycerides, each of the highest purity obtainable, were purchased from a firm specializing in pure organic chemicals. Pure virgin olive oil was also purchased. The oil was stored at about 4°C.

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The percentage composition of the diets which were fed to the rats was as follows: Diet 1 — casein 18.6; salts 4.0; dextrose 75.4; yeast vitamins 2.0; and no fat. Diet 2 — casein 20.0; salts 4.3; dextrose 68.7; yeast vitamins 2.0; and fat 5.0. To 1 kilo of each diet was added 100 ml. of cod liver oil concentrate.[1]

EXPERIMENTAL PROCEDURE

Each diet containing 5% of a mixture of olive oil and a definite proportion of a fatty acid or triglyceride was fed to eight mature male rats for 10 days. After a preliminary period of 3 days, the feces were collected for 7 days from each rat and the quantity of feed consumed by each was determined. Each rat was then changed to a fat-free diet and, after a preliminary period of 3 days, the feces were collected for 7 days. The feces were dried to constant weight at 100°C. and the fat content was determined as previously described by the writers ('42). When the diet contained fatty acids, the digestive coefficient was calculated as the percentage of the total fatty acids. The digestive coefficient was calculated for each of the eight rats on each diet, but only the average value is reported.

EXPERIMENTAL RESULTS

The results of the digestion tests with two lots of olive oil and with mixtures of olive oil and different proportions of stearic, myristic, and lauric acids are shown in table 1. The melting points of the pure fatty acids and of the mixtures are shown also. Two lots of olive oil were used, as the first was insufficient.

The results of the tests with the mixtures of olive oil and saturated fatty acids show that stearic acid depressed the digestibility of the mixture only slightly less than the proportion of stearic acid present. The digestive coefficients for stearic acid alone have been calculated on the assumption that the reduction in digestibility was due entirely to unabsorbed stearic

[1] 100 ml. of an ethereal extract from saponified cod liver oil was added to 1 kg. of the diet and the mixture was agitated until the ether had evaporated.

TABLE 1

Digestibility by male rats of mixtures of certain saturated fatty acids and olive oil when the diets contained 5% of fat.

DESCRIPTION OF FAT MIXTURE	MELTING POINT OF PURE FATTY ACIDS °C	MELTING POINT OF MIXTURE °C	DIGESTIVE COEFFICIENT OF MIXTURE Average[1] %	Range %	CHANGE IN DIGESTIBILITY COMPARED WITH OLIVE OIL %	CALCULATED DIGESTIBILITY OF PURE FATTY ACIDS %
Olive oil no. 4085[2]			97.4	94.1– 99.3		
Olive oil no. 4095			99.5	99.1–100.0		
Stearic acid 5%, olive oil 95%	69	43	92.6	90.5– 95.5	– 4.8	9.4
Palmitic acid 5%, olive oil 95%	63	37	94.2	91.9– 96.6	– 3.2	39.6
Myristic acid 5%, olive oil 95%	53		99.1	97.6–100.0	+ 1.7	
Lauric acid 5%, olive oil 95%	44		98.0	97.3– 99.0	+ 0.6	
Stearic acid 10%, olive oil 90%		51	88.3	85.0– 91.2	– 9.1	13.3
Palmitic acid 10%, olive oil 90%		44	90.8	87.6– 94.2	– 6.6	37.1
Myristic acid 10%, olive oil 90%		32	97.6	95.0– 99.9	+ 0.2	
Lauric acid 10%, olive oil 90%			98.3	97.1– 99.7	+ 0.9	
Stearic acid 15%, olive oil 85%		55	85.0	79.4– 89.5	– 12.4	21.0
Palmitic acid 15%, olive oil 85%		48	86.6	82.4– 89.8	– 10.8	31.2
Myristic acid 15%, olive oil 85%		39	97.1	93.8–100.0	– 0.3	
Lauric acid 15%, olive oil 85%			97.2	96.6– 98.5	– 0.2	
Stearic acid 25%, olive oil 75%		59	76.5	71.1– 80.1	– 20.9	19.6
Palmitic acid 25%, olive oil 75%		53	77.6	75.0– 82.4	– 19.8	23.8
Myristic acid 25%, olive oil 75%		44	92.7	88.4– 97.0	– 4.7	81.9
Lauric acid 25%, olive oil 75%		32	92.6	82.5– 96.3	– 4.8	81.5
Stearic, palmitic, myristic, and lauric acids, 2.5% each; olive oil 90%			93.5	90.4– 97.0	– 6.0	

[1] Each digestive coefficient represents the average value obtained with eight rats.

[2] Olive oil no. 4085 was used in all mixtures except the last one.

acid. To obtain these figures, the percentage of fatty acid in each mixture was calculated as the percentage of the total fatty acids, since the digestive coefficients were calculated on that basis.

The results of the digestion tests with the mixtures of palmitic acid and olive oil show that each mixture had a somewhat higher digestive coefficient than a similar mixture of stearic acid and olive oil. The approximate digestive coefficients for palmitic acid alone were calculated in the same manner as for stearic acid.

Myristic and lauric acids were very thoroughly absorbed when the percentage of acid in the mixture did not exceed 15%; but when the proportion was increased to 25%, the digestibility was materially lower. The approximate digestive coefficients for myristic and lauric acids alone are shown only for the highest level of intake.

The digestibility of the mixture of 2.5% each of stearic, palmitic, myristic, and lauric acids and olive oil no. 4095 was lower than might have been expected from the results previously reported in table 1. This mixture had a digestive coefficient six points lower than the olive oil used although the mixture contained only 2.5% each of stearic and palmitic acids.

The results of the digestion tests with the mixtures of triglycerides and olive oil are shown in table 2. The results with tristearin show that this triglyceride, whether present singly or together with other triglycerides in a mixture with olive oil, depressed the digestibility of the fat nearly as much as the percentage of tristearin present. When the mixtures contained 5 and 10% of tristearin, the approximate digestive coefficients of the tristearin alone were calculated on the assumption that the reduction in digestibility was due entirely to unabsorbed tristearin.

The results of the tests with the mixtures containing tripalmitin indicate that this triglyceride was much more digestible than tristearin. The approximate digestive coefficients for tripalmitin alone, when the mixtures contained 5 and 10% of this triglyceride, are shown.

TABLE 2

Digestibility by male rats of mixtures of certain saturated triglycerides and olive oil when the diets contained 5% of fat.

DESCRIPTION OF FAT MIXTURE	MELTING POINT OF TRIGLYCERIDE °C	MELTING POINT OF MIXTURE °C	DIGESTIVE COEFFICIENT OF MIXTURE		CHANGE IN DIGESTIBILITY COMPARED WITH OLIVE OIL %	CALCULATED DIGESTIBILITY OF TRIGLYCERIDE %
			Average [1] %	Range %		
Olive oil no. 4085 [2]			97.4	94.1– 99.3		
Olive oil no. 4095			99.5	99.1–100.0		
Tristearin 5%, olive oil 95%	70	55	92.7	91.3– 94.4	– 4.7	6.0
Tripalmitin 5%, olive oil 95%	65	45	95.8	91.2– 99.3	– 1.6	84.0
Trimyristin 5%, olive oil 95%	56		96.4	93.5– 99.8	– 1.0	
Trilaurin 5%, olive oil 95%	48		98.2	94.8– 99.9	+ 0.8	
Tristearin 10%, olive oil 90%		59	89.2	88.0– 91.3	– 8.2	8.0
Tripalmitin 10%, olive oil 90%		51	95.6	92.9– 97.2	– 1.8	82.0
Trimyristin 10%, olive oil 90%		42	99.0	97.8–100.0	+ 1.6	
Trilaurin 10%, olive oil 90%			99.9	99.5–100.0	+ 2.5	
Tristearin 5%, trimyristin 5%, olive oil 90%		54	95.3	93.9– 96.8	– 4.2	
Tristearin 5%, trilaurin 5%, olive oil 90%		53	95.2	91.1– 96.6	– 4.3	
Tripalmitin 5%, trimyristin 5%, olive oil 90%		42	96.3	93.7– 98.4	– 3.2	
Tripalmitin 5%, trilaurin 5%, olive oil 90%		40	97.7	96.6– 98.5	– 1.8	
Tristearin, tripalmitin, trimyristin, and trilaurin 2.5% each; olive oil 90%			96.9	94.1– 98.6	– 2.6	

[1] Each digestive coefficient represents the average value obtained with eight rats.

[2] Olive oil no. 4085 was used in the first two series of digestion tests and sample no. 4095 was used in the third series.

The mixtures containing 5 or 10% each of trimyristin or trilaurin were as digestible as the olive oil alone. Under these conditions each triglyceride was practically 100% digestible.

A comparison between the results obtained with the fatty acids and those with the corresponding triglycerides indicates that both tristearin and stearic acid were very poorly absorbed, whereas tripalmitin appeared to have been much better absorbed than palmitic acid. Myristic and lauric acids and the corresponding triglycerides were utilized equally well.

SUMMARY

Experiments were conducted with mature male rats to determine the digestibility of pure stearic, palmitic, myristic and lauric acids and of the corresponding triglycerides. Each fatty acid was mixed in the proportions of 5, 10, 15, and 25% with pure olive oil, and each triglyceride was mixed in the proportions of 5 and 10%. The fat mixture constituted 5% of the diet. The following results were obtained:

Stearic acid was very poorly absorbed at each level of intake, the approximate digestive coefficients ranging from 9.4 to 21%.

Palmitic acid was utilized somewhat more efficiently than stearic acid, the approximate digestive coefficients ranging from 23.8 to 39.6%.

Myristic and lauric acids were practically 100% digestible when the fat mixtures contained 5, 10, or 15% of either acid, but the digestibility was somewhat lower when the mixture contained 25% of acid.

Tristearin was very poorly utilized, the approximate digestive coefficients being 6 and 8% when the fat mixtures contained 5 and 10% of the triglyceride.

Tripalmitin was much more digestible than tristearin, the approximate digestive coefficients being 84 and 82% when the fat mixtures contained 5 and 10% of the triglyceride.

Trimyristin and trilaurin were very thoroughly absorbed.

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THE EFFECT OF COOKING WITH AND WITHOUT SODIUM BICARBONATE ON THE THIAMINE, RIBOFLAVIN, AND ASCORBIC ACID CONTENT OF PEAS [1]

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Sodium bicarbonate has been employed in the cooking of vegetables, largely because of the decreased cooking time required and because its use is attended by retention of the normal color of green vegetables. On the other hand, the idea has become fairly general among nutritionists that the addition of sodium bicarbonate will result in a large destruction of certain members of the B complex (chiefly thiamine and riboflavin) as well as of vitamin C. In fact, the current publicity campaign for better nutrition has stressed that the use of soda should be avoided in cooking vegetables.

Halliday ('32a) found that the thiamine in protein-free milk was 70 to 80% destroyed by cooking for 1 hour at a pH of 10, and completely destroyed by heating for 4 hours at this pH. The rate of destruction was much less at a pH of 4.3 or 7.0 where the maximum loss was 40%. Lantz ('38) found as much as 50% loss in the thiamine of Pinto beans soaked 16 hours in distilled water following by cooking $2\frac{3}{4}$ hours. Somewhat larger losses occurred when a 0.5% solution of sodium bicar-

bonate was used for the preliminary 16-hour soaking period although the results on the bioassay group receiving 0.2 gm. of dried peas actually were considerably better with the soda group. It is also uncertain how much loss of thiamine can be traced to the procedure used in drying the beans, as Mickelsen, Waisman and Elvehjem ('39) have found that an appreciable destruction of thiamine occurs in drying even when a vacuum is employed if the temperature exceeds 70° C. Aughey and Daniel ('40) also have found a slightly greater destruction of thiamine in peas cooked in water alone and in water containing sodium bicarbonate, 11% of the original thiamine being in the water in both cases while 80% and 67%, respectively, were retained in the peas. An identical time of cooking (12 minutes) was used in both instances.

Although riboflavin is extremely thermostable, it is especially sensitive to light. Conner and Straub ('41) and Williams and Chedelin ('42) have demonstrated the rapid destruction of this vitamin by sunlight. Halliday ('32b), in experiments carried out with the thiamine tests by an identical plan described earlier, also showed that the destruction of riboflavin was somewhat greater when heated at a pH of 10 than obtains at a pH of 4.3 or even at one of 7.0. Lantz ('39) found that the availability of riboflavin in Pinto beans is influenced somewhat by the method of cooking which included the use of sodium bicarbonate, but the differences were not great.

While it has been recognized for a long time that vitamin C is somewhat thermolabile, it is now evident that the most rapid destruction occurs when oxidation is brought about in the presence of copper or silver salts. However, Sherman ('41) in discussing the effect of soda in cooking, states the following about the extent of destruction:

> "Not necessarily all of it; but always more of the vitamin C is destroyed when soda is added than when it is not. The more soda added the more vitamin destroyed; this is true whether or not the change in pH caused is such as to shift it over the neutral point of pH = 7".

The present tests were designed to get additional data on the relative destruction of thiamine, riboflavin and vitamin C of peas when cooking was carried out with water alone or with water to which sodium bicarbonate was added. The earlier data are not entirely applicable to the cooking of fresh vegetables as pH values as high as 10 will not occur when the quantities of sodium bicarbonate used in cooking are employed; moreover, most of the earlier investigators have made comparisons of the effect of cooking with and without soda on the basis of identical times of cooking rather than on the periods required to complete the cooking under the two procedures.

METHODS OF ANALYSIS

Thiamine was determined by a modification of the yeast fermentation method of Schultz, Aiken, and Frey ('42). This procedure was changed by weighing the water forced over from the gasometers into bottles during the course of the fermentation rather than by reading off the volumes. Also as containers for the reaction mixtures, 250 ml. centrifuge tubes connected with the tubes to the gasometers by spherical joints were found more convenient and to obviate leakage. The reliability of the above method of analysis was confirmed by comparing the thiamine contents of peas dried at 60°C. in a vacuum oven after cooking with water alone or water containing sodium bicarbonate as determined by the yeast fermentation method and the bioassay procedure of Kline, Hall and Morgan ('41).

Riboflavin was estimated by the microbiological method of Snell and Strong ('39). In order to obtain the full response, digestion with takadiastase apparently is unnecessary, as identical results were obtained with and without the preliminary treatment. That our results represented riboflavin is evident from the fact that activity of the pea suspension was completely destroyed by photolysis.

The technic of Loeffler and Ponting ('42) was used for the determination of vitamin C. Not only were uniform results

obtained when vitamin C was determined promptly after preparation of pea suspensions, but also it was demonstrated that ascorbic acid added to such suspensions could be quantitatively accounted for.

Peas were cooked in a covered aluminum saucepan or in a similar pyrex dish with a cover. In these experiments, 85 gm. of peas were cooked with 180 ml. of water. When sodium bicarbonate was used, 0.22 gm. was added. In preliminary tests the periods of time required for peas to become tender, when cooked in water alone or water plus sodium bicarbonate, were determined. The periods required for fresh peas were 17 and 8 minutes, respectively, while with frozen peas, the values were 6 and 4 minutes. In all cases the peas were added to the water after it had been brought to a boil.

After cooking, the peas were filtered from the cooking water, transferred to a Waring blender, fresh water added and the peas macerated to a fine susupension. Analyses were made both on the cooking water and on the suspension of the peas. The extent of retention of the green color in the peas was established by comparing the color of the mashed pulp with standard color plates (Maerz and Paul, '30). The pH was determined by the use of the Beckman pH meter.

The peas were purchased in various local markets. The frozen peas consisted of one variety where plate freezing was employed and several varieties where tunnel freezing was used.

RESULTS

A summary of the results on the thiamine content of peas as influenced by the method of cooking is given in table 1.

The thiamine content of the raw fresh peas averaged 333 µg. per 100 gm. while that of the two types of frozen peas gave mean values of 408 and 351 µg. These fall within the values listed by Sherman ('41). The addition of sodium bicarbonate increased the pH of the water somewhat over 1 pH unit, the values found after cooking being between 8.70 and 8.84. In the frozen peas prepared by tunnel freezing and in the fresh peas, identical quantities (about 80%) of the original thiamine

were retained after cooking the peas either with or without sodium bicarbonate. However, slightly greater losses were incurred in the thiamine which had been leached into the cooking water when soda was employed, but the destruction only amounted to 8 and 4% of the total amount present in the frozen and fresh peas, respectively.

TABLE 1

The thiamine contents of frozen and fresh peas before and after cooking in water alone or water containing sodium bicarbonate.

TYPE OF PEAS AND METHOD OF COOKING	NO. OF TESTS	pH OF WATER AFTER COOKING	THIAMINE IN MICROGRAMS PER 100 GM.			PER CENT RETAINED IN PEAS	PER CENT LOST
			In peas	In cooking water	Total		
Frozen type I [1]							
Uncooked	3				408		
Water-cooked	3	7.66	326	90	416	79.8	0
NaHCO₃-cooked	3	8.77	330	44	374	80.8	8.3
Frozen type II [1]							
Uncooked	1				351		
Water-cooked	1		238	102	340	67.8	3.1
NaHCO₃-cooked	1	8.70	193	25	218	55.0	37.9
Fresh							
Uncooked	4				333		
Water-cooked	4	7.29	257	78	336	77.2	0
NaHCO₃-cooked	4	8.84	258	63	321	77.4	3.6
Fresh (overcooked)							
Uncooked	1				185		
Water-cooked	1	7.50	67	34	101	36.3	45.4
NaHCO₃-cooked	1	9.38	59	19	78	31.9	59.6

[1] Type I represents a brand of peas prepared by tunnel freezing; and type II one prepared by plate freezing.

In peas frozen by the plate process where many of the cell membranes were ruptured, the loss was greater in the peas cooked with sodium bicarbonate. The thiamine retained in the peas cooked in water was 68% while that remaining in those cooked with sodium bicarbonate was only 55%. Approximately 38% of thiamine was lost in the soda-cooked peas and this was largely due to the greater destruction of that leached into the

water. In another series of tests when fresh peas were over-cooked and many of the cell membranes ruptured, the loss of thiamine also was greater than in the earlier tests. Only somewhat over 30% of the original thiamine was retained in the peas and the loss amounted to 45 and 60% in the water- and $NaHCO_3$-cooked samples, respectively.

Identical results were found for the thiamine content of dried peas as determined by the fermentometer method and by bioassay. Thus, the average thiamine content for the dried peas which had been cooked in water was 1050 and 1070 µg. per 100 gm., respectively, when determined by the fermentometer method and by bioassay. The results on the peas which had been cooked in sodium bicarbonate were 1345 and 1330 µg., respectively. This would indicate that no biologically inactive products which stimulate yeast growth and are not destroyed by sodium sulfite are formed in peas during cooking with water alone or with water and bicarbonate.

The results of cooking on the riboflavin content of fresh and frozen peas are given in table 2.

The riboflavin content of the various samples of frozen peas varied from 171 to 200 µg. per 100 gm., while the limits of values for fresh peas were practically identical (168 to 203). These values are slightly lower than the figures reported by Sherman ('41) where the limits are given as 200 to 250 µg.

In all cases 64 to 70% of the riboflavin remained in the peas after cooking, and the figures are identical regardless of method of cooking. Practically no destruction of vitamin B_2 occurred with the fresh peas and only a slight decrease in the frozen peas regardless of the procedure used in cooking. No differences in the riboflavin content of peas cooked in the dark (aluminum pan) and in the light (pyrex dish) were noted.

The effects of cooking on the ascorbic acid content of fresh peas and three types of frozen peas are reported in table 3.

The ascorbic acid content of the fresh peas averaged 23.7 mg. per 100 gm., which is in the upper range given by Sherman ('41). The values on frozen peas are consistently lower. It is possible that this may be due to the lower content of vitamin

TABLE 2

The riboflavin content of fresh, frozen, and dried cooked peas as affected by cooking with water alone and with added sodium bicarbonate.

TYPE OF PEAS AND METHOD OF TREATMENT	NO. OF TESTS	TYPE OF CONTAINER IN COOKING	RIBOFLAVIN IN MICROGRAMS PER 100 GM.			PER CENT RETAINED IN PEAS	PER CENT LOST
			In peas	In cooking water	Total		
Frozen type I [1]							
Uncooked	1				171		
Photolyzed [2]	1				0		100
Uncooked	1				189		
Water-cooked	1	Pyrex	122	42	164	64.8	13.1
NaHCO₃-cooked	1	Pyrex	127	46	163	67.0	13.8
Uncooked	1				200		
Water-cooked	1	Aluminum	141	46	187	70.5	6.5
NaHCO₃-cooked	1	Aluminum	141	45	186	70.5	7.5
Fresh peas							
Uncooked	1				168		
Takadiastase [3]	1				170		
Uncooked	2				203		
Water-cooked	2	Pyrex	132	73	205	65.0	0.0
NaHCO₃-cooked	2	Pyrex	131	73	204	64.6	0.0
Water-cooked	2	Aluminum	130	68	198	64.1	2.5
NaHCO₃-cooked	2	Aluminum	135	65	200	66.6	1.5
Dried peas [4]							
Water-cooked	1	Pyrex			468		
NaHCO₃-cooked	1	Pyrex			492		

[1] Type I represents a brand of peas prepared by tunnel freezing, and type II one prepared by plate freezing.

[2] An alkaline solution was subjected for 8 hours to the light from a 75-watt lamp at 30 cm.

[3] 0.1 gram of takadiastase (Merck) was added to pea suspension; they were incubated for 3 hours, autoclaved, diluted and added to tubes for assay.

[4] Dried peas used in bioassay test on thiamine. Before drying, these had been cooked with water or water containing NaHCO₃ as in other tests.

TABLE 3

The ascorbic acid content of fresh and frozen peas as affected by cooking with water alone and with added sodium bicarbonate.

TYPE OF PEAS AND METHOD OF TREATMENT	NO. OF TESTS	ASCORBIC ACID IN MG. PER 100 GM.			PER CENT RE-TAINED IN PEAS	PER CENT LOST
		In peas	In cooking water	Total		
Frozen peas, type I [1]						
Uncooked (brand 1)	1			20.6		
Uncooked (brand 2)	1			19.8		
Uncooked (brand 1)	1			18.1		
Water-cooked	1	11.0	4.3	15.3	60.7	15.5
NaHCO₃-cooked	1	11.0	3.7	14.7	60.7	18.8
Uncooked (brand 2)	1			18.0		
Water-cooked	1	10.1	4.8	14.9	56.2	17.2
NaHCO₃-cooked	1	10.3	4.0	14.3	57.2	20.5
Frozen peas, type II [2]						
Uncooked [3]	3			12.2		
Uncooked [4]	1			18.2		
Water-cooked	1	8.2	7.3	15.5	45.1	14.8
NaHCO₃-cooked	1	8.1	7.4	15.5	45.0	14.8
Fresh peas						
Water-cooked [5]	1	12.9	13.7	26.6		
NaHCO₃-cooked	1	15.0	11.2	26.2		
Uncooked	3			23.7		
Water-cooked	3	11.6	10.3	21.9	48.9	7.6
NaHCO₃-cooked	3	12.9	10.5	23.4	54.4	1.3

[1] Frozen by tunnel process.

[2] Frozen by plate process.

[3] Frozen peas were allowed to thaw before being weighed and only peas with intact skins were used.

[4] Frozen peas weighed without thawing.

[5] No value on uncooked peas.

C in the fresh peas from which the frozen peas were prepared; on the other hand, it may be that some vitamin C is destroyed in the preparation for freezing or in the storage. It is well-known that peas prior to freezing are treated with boiling water for short periods after mechanical shucking to remove undesirable residues. Because of the readier solubility of vitamin C than of thiamine or riboflavin, it is possible that some is leached out by this treatment. The proportion of ascorbic acid remaining in the pea after cooking varies from 45 to 60%. No greater leaching out or destruction of the vitamin C obtains in the peas cooked in water containing bicarbonate of soda than in those cooked in water alone.

The greater retention of the green color in peas cooked with sodium bicarbonate was indicated from the deeper green color of the pulp. After cooking in water the color compared with plate 20, no. 1 or 3, L, while after the use of sodium bicarbonate the pulp matched plate 19, 5 or 6, L, which is a greener shade, but contains less gray (Maerz and Paul, '30).

DISCUSSION

The addition of sodium bicarbonate in the proportion of 0.22 gm. ($\frac{1}{16}$ teaspoonful) to 180 ml. of water (1 cup) resulted in a decrease in the time required for cooking fresh peas (from 17 to 8 minutes) and frozen peas (from 6 to 4 minutes). There was only slightly greater destruction of thiamine under these conditions when sodium bicarbonate was added and this was due to the greater loss in that leached out of the peas; however, the amount still remaining in the peas at the conclusion was the same by both methods of cooking. On the other hand, the loss was greater when fresh peas were overcooked to the extent that they started to fall apart and in one sample of frozen peas in which the peas had been partially crushed in the processing. Both with water alone and with water containing sodium bicarbonate, much smaller quantities of thiamine remained in the peas.

The losses of riboflavin were under 15% in all tests and in four of the six series they were practically zero. There was no

greater destruction in the soda-cooked series than in those cooked in water alone; moreover, the retention of riboflavin in the peas, which varied from 64 to 70%, was similar when both methods of cooking were employed.

Vitamin C in general rather than thiamine or riboflavin was found to be more readily soluble in the cooking water, the amounts retained in the peas varying from 61 to 45%. The total destruction of the ascorbic acid was usually under 20% and here again there was no difference in the two methods of cooking. The destruction was much less in the fresh peas than in the frozen samples. In the former case only 1% destruction resulted in the peas cooked in sodium bicarbonate, while a loss of 8% was found in those boiled in tap water.

One reason why these results do not show the destructive effect of sodium bicarbonate that is reported in some of the earlier studies, is that the alkalinity employed was less. Whereas Halliday used a pH of 10, the values obtained in our tests were usually under a pH of 9. Secondly, the time of cooking used by some investigators has been the same for the sodium bicarbonate tests and those where water alone was employed. One of the chief advantages in the use of soduim bicarbonate is the shorter cooking time that its addition makes possible. In these tests the time necessary for cooking the peas sufficiently tender to be appetizing has been employed. Although thiamine, riboflavin and ascorbic acid are undoubtedly more thermolabile at the higher pH values, this tendency is compensated by the shorter cooking time possible when sodium bicarbonate is added. Also if the same length of cooking time is used with and without sodium bicarbonate, there is likely to be a greater destruction in the latter case because the peas begin to disintegrate. Any time when the intact membrane of the pea becomes ruptured with or without sodium bicarbonate, a markedly increased destruction of these water-soluble vitamins results.

The loss by leaching into the cooking water is least with thiamine, somewhat more with riboflavin and greatest with ascorbic acid. This may be because the two B vitamins are

largely non-diffusible since they are combined with protein while ascorbic acid is almost entirely free. The ready leaching of ascorbic acid may possibly account for the lower value found in frozen, as compared with fresh, peas.

The destruction of the thiamine, riboflavin, and ascorbic acid is undoubtedly considerably retarded by the buffering effect of the pea proteins. Also the cell membranes probably prevent any appreciable diffusion of the sodium bicarbonate to the inside of the cell. The protective effect on the peas is shown by the far greater destruction which occurs when 12.5 mg. of ascorbic acid are cooked with 160 ml. of water at various pH levels; when cooked for 17 minutes, 46.8% was destroyed in tap water, 13.6% at a pH of 4.3, and 58.2% in sodium bicarbonate for 8 minutes.

Further experiments are necessary to establish how general may be the application of the results obtained herein. It would seem advisable to re-investigate the destructive effect of sodium bicarbonate in the cooking of a wide variety of vegetables, making use of the more accurate procedures now available for the determination of the vitamins.

SUMMARY

The effect of cooking fresh and frozen peas in water alone and in water containing sodium bicarbonate on the thiamine, riboflavin and vitamin C content has been studied.

It has been found that the addition of 0.22 gm. of $NaHCO_3$ to 180 ml. of water in the cooking of 85 gm. of fresh peas lowers the time necessary for cooking from 17 to 8 minutes; of frozen peas, the decrease is from 6 to 4 minutes.

The following average values were found per 100 gm. of fresh peas: thiamine, 333 µg.; riboflavin, 168 to 203 µg.; and vitamin C, 23.7 mg. In frozen peas the values were similar with thiamine (351 to 408 µg.) and riboflavin (171 to 200 µg.), but were somewhat lower with ascorbic acid (12.2 to 20.6 mg.).

Approximately 80% of the thiamine, 65% of riboflavin, and 45 to 60% of ascorbic acid were retained in the peas after

cooking. No differences resulted from the addition of sodium bicarbonate to the cooking water.

When the vitamin content remaining in the peas was added to that of the cooking water, the recovery of these three vitamins was found to be practically complete. Similar results were obtained with respect to frozen peas, as far as thiamine and riboflavin are concerned. However, the destruction of vitamin C amounted to between 15 and 20% with the frozen peas, and this was not influenced by the method of cooking.

When fresh peas and one brand of frozen peas which were frozen by the plate process were overcooked to the extent that they started to disintegrate, the losses of thiamine from the peas were considerably greater and the recovery was much less. Under these conditions the destructive effect of sodium bicarbonate was found to be somewhat greater.

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THE B VITAMINS IN HONEY [1]

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Honey is a widely used food — undoubtedly due to its sweetness, pleasant taste and aroma. Since honey is a natural substance and is food for the honeybee, there has been much speculation as to its value in the nutrition of man. Honey is prepared from the nectar of flowers by worker bees and stored in the honeycomb. Although honey is derived primarily from nectaries of flowers, we cannot overlook the part the honeybee plays in storing the nectar in the hive and in processing it to its final form. Thus honey should be considered the product of the bee processed from a plant product.

The analysis of honey has shown that it consists of approximately 75–80% sugar, mainly invert sugar, which is a conversion product of sucrose. Schuette et al. ('32, '37, '38, '39) have shown the presence of mineral elements in honey. Notable among these are iron, copper, sodium, potassium, manganese, calcium, magnesium, and phosphorus, all of which have been shown to be essential to good nutrition of animals. Since honey is basically derived from the flower and moreover is a food for the bee, much speculation has arisen as to its vitamin content, especially because great emphasis has been placed on the importance of vitamins in human nutrition. It seems that this problem has interested workers for two decades, many of whom have reported the absence of vitamins A, B, C, D, and E

in honey. These results may be interpreted in the light of the methods employed, which have been much improved in recent years.

Recently chemical determinations of vitamin C in honey have been made by Griebel ('38). He reported 160–280 mg. ascorbic acid per 100 gm. of mint honey and 7–22 mg. per 100 gm. of other honeys. Griebel and Hess ('39) identified the strong reducing substance in thyme and mint honeys as ascorbic acid. They reported 311.2 mg. and 102.6 mg. of this vitamin per 100 gm. of the respective honeys. Buckwheat honeys contained from 7.36–18.6 mg. ascorbic acid per 100 gm. They (Griebel and Hess, '40) determined also the ascorbic acid content of the nectar of the above plants. It was shown that there is a considerable loss of vitamin C when nectar is converted into honey. Schuette and Kaye ('42) investigated a series of honeys and found 0.55–20.9 mg. of vitamin C per 100 gm. The degree of destruction of vitamin C in honey was also studied. It was noted that there was a decrease in the total ascorbic acid content of honey after pure ascorbic acid was added. The loss was greatly increased in the honeys placed in diffused light.

Haydak and Palmer ('42 a) reported the vitamin content of honeys of varied nectar source and origin. They reported the following values per 100 gm. of honey: thiamine, 2.1–9.1 µg.; riboflavin, 35–145 µg.; pyridoxine, 210–480 µg.; pantothenic acid, 25–192 µg.; nicotinic acid, 4–94 mg.; ascorbic acid, 0.6–5.4 mg.

The authors of this paper will attempt to give a clear picture of the vitamins in honey obtained by the improved methods of vitamin assay. These include the more recent michrochemical and microbiological methods, some of which have been modified in the University of Wisconsin. Preliminary investigations did not reveal the presence of the fat-soluble vitamins; therefore only the contents of water-soluble vitamins were determined. Together with numerous samples of honey we have analyzed pollen and royal jelly, two natural substances also serving as food for bees.

EXPERIMENTAL

The honeys under investigation were obtained from the collection in the Laboratory of Foods and Sanitation of the Chemistry Department of the University of Wisconsin, from local grocery stores, and from beekeepers in various localities of the United States. Many of these honeys were received in the comb and extracted in the laboratory by straining through layers of cheesecloth. The wax was washed with distilled water and dried for further analysis.

The nicotinic acid assays were made by the microbiological method of Snell and Wright ('41). Samples were prepared as follows: 25 gm. of honey were diluted to 75 ml. with distilled water, autoclaved for 15 minutes at 15 pounds pressure, and then diluted to the necessary volume. It was found that aqueous extraction gave the same results as acid or alkali. The latter tended to produce excessive caramelization of the sugars in the honey. The effect of the large percentage of sugar on the assay method and also the effect of caramelization were investigated. The sugar that was being introduced by the sample of honey had no apparent effect on the assay, but the caramelization which resulted from acid or alkali treatment interfered very much with the determination of the end-point in the titration of the lactic acid produced. Recovery of added nicotinic acid ranged from 90% to 110%.

Pantothenic acid was determined by the microbiological method of Strong, Feeney and Earle ('41). Preparation of the sample was carried out as follows: 25 gm. of honey were diluted to 75 ml. and neutralized to pH 7.0 with NaOH. It was autoclaved for 15 minutes at 15 pounds pressure and diluted to the necessary volume. Enzyme digestion of the honey with clarase did not produce a significant change in the results. Ether extraction of the diluted honey did not remove any stimulatory or inhibitory substances. Recovery of added pantothenic acid ranged from 90% to 110%. Values are expressed in micrograms of calcium pantothenate per 100 gm.

Riboflavin was determined by the microbiological method of Snell and Strong ('39). Preparation of the sample was as follows: 25 gm. of honey were diluted to·75 ml. with distilled water and autoclaved for 15 minutes at 15 pounds pressure. The use of acid was avoided for the reasons explained for the nicotinic assay. Enzyme digestion with clarase did not alter the value significantly. It should be mentioned that the natural pH range of honey is 4–5; therefore the honey dilution was acid during the autoclaving, thereby reducing any possible destruction of the riboflavin. Ether extraction of the honey dilution did not remove any stimulatory or inhibitory substances.

Thiamine was determined by the Hennessy-Cerecedo thiochrome method as modified by the Research Corporation Committee on the Thiochrome Method (Hennessy, '42). A Coleman photofluorometer was used to measure the fluorescence of the thiochrome produced by the oxidation of the thiamine. This instrument can accurately determine 0.02 µg. of thiamine. Recovery of added thiamine ranged from 85% to 105%. Values are expressed in micrograms of thiamine-hydrochloride.

Pyridoxine was determined by the microbiological method of Atkin, Schultz, Williams, and Frey ('43). In this method the response of a strain of yeast to pyridoxine is measured by turbidity readings. The range of sensitivity of the assay is 5 to 40 mµg. Enzyme digestion was used to release any bound pyridoxine. Recovery of added pyridoxine ranged from 95% to 105%. Values are expressed in micrograms of pyridoxine-hydrochloride per 100 gm.

Biotin was determined by the microbiological method of Snell, Eakin, and Williams ('40), using yeast and measuring the growth of the organism by turbidity readings. The assays were conducted by Miss Josephine Gardner of the Biochemistry Department of the University of Wisconsin.

Folic acid was determined by the method of Mitchell and Snell ('41), using the streptococcus lactis organism. The assays were conducted by Mr. T. D. Luckey of the Biochemistry Department of the University of Wisconsin. The folic acid

values are expressed in this paper as micrograms of folic acid based on a solubilized liver standard with a potency of 40,000.

The samples of pollen which were analyzed were obtained from Professor Farrar of the Department of Economic Entomology of the University of Wisconsin. One sample of pollen was a mixture from an area around Madison, Wisconsin. The other sample was a mixture of pollen from Baton Rouge, Louisiana. The sample of royal jelly was obtained in a dry form from Dr. H. B. Parks of the Texas Agricultural Experiment Station.

RESULTS AND DISCUSSION

Table 1 gives the results of analyses of forty samples of honeys from different regions of the United States. Although the source of the honey is also listed, it is understood that honey is usually a mixture of nectars. The floral source listed, however, is the predominant one. An examination of the values obtained for the five members of the B-complex showed great variation among the samples. The greatest variation was in the nicotinic acid content. In one instance, a sample of honey contained nine times as much nicotinic acid as another. The least variation occurred in the thiamine and pyridoxine values, but even here the variation was as much as fivefold. An attempt was made to correlate the vitamin content with floral source, origin, and color-grade. This proved fruitless for we did not have a sufficiently large number of samples of honey of one locality to enable us to say that one set of data is typical of that source. The wide variation of the vitamin contents precluded any attempt to correlate the values with the color-grade of the honeys.

Table 2 presents a summary of the vitamin content of the honeys examined. It also shows the differences in the vitamin content of the old honeys as compared with the new samples. In the course of the investigation for pantothenic acid, it became apparent that the pantothenic acid values for a large number of honeys were lower than those of the rest. It was found that the group of honeys that had been obtained from

TABLE 1
Summary of vitamin assays of honey.
All values are in micrograms per 100 gm.

ORIGIN	FLORAL SOURCE	RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID	THIAMINE	PYRIDOXINE
Alabama	cotton	60	360	590	12.0	17.5
Arkansas	holly blossom	27	60	140	1.4	...
California	alfalfa	50	16	240	6.2	8.4
California	alfalfa	25	20	110	2.9	6.4
California	mesquite	14	24	93	3.5	...
California	orange blossom	9	9	96	2.5	6.3
California	sage	40	60	100	4.6	9.2
California	spikeweed	10	15	88	2.6	8.3
California	tamarisk	24	9	105	4.3	10.2
California	thistle	45	19	137	3.3	...
California	white sage	13	9	80	3.4	5.3
California	white sage	12	17	120	2.4	...
California	wild buckwheat	20	17	181	4.2	14.0
California	wild sage-buckwheat	21	17	135	4.2	10.2
Florida	orange blossom	30	60	95	3.8	7.0
Florida	tupelo	20	27	104	5.5	3.9
Florida	tupelo	15	13	100	3.0	8.9
Georgia	Mexican clover	15	13	75	2.2	7.8
Georgia	titi	18	20	63	2.9	4.4
Illinois	orange blossom	28	27	110	4.8	14.2
Illinois	sweet clover	20	46	80	3.4	5.5
Illinois	unknown	11	9	110	2.2	...
Iowa	sweet clover	22	40	110	4.2	9.6
Louisiana	white dutch clover	32	110	176	3.9	27.7
New York	buckwheat	24	63	123	4.2	12.6
Ohio	mixed flowers	35	116	110	5.5	11.7
Ohio	sweet clover	12	50	90	3.9	7.2
Texas	horse mint	64	12	600	4.3	...
Utah	sweet clover	20	32	72	2.8	4.6
West Virginia	sweet clover	19	32	150	4.2	8.2
Wisconsin	sweet clover	7	61	225	5.5	12.2
Wisconsin	sweet clover	20	65	98	2.8	5.2
Wisconsin	sweet clover	23	38	100	3.4	9.0
Wisconsin	sweet clover	19	52	89	2.5	5.7
Wisconsin	sweet clover	14	20	270	4.4	5.0
Wisconsin	white and sweet clover	15	35	85	5.6	10.6
Wisconsin	white and sweet clover	12	57	95	3.8	8.9
Wisconsin	white and sweet clover	33	48	200	4.2	7.5
Unknown	orange blossom	30	20	119	2.2	6.4
Unknown	sweet clover	30	44	75	3.7	4.6

the Chemistry Department contained 50% less pantothenic acid than those received from various beekeepers. The former were of the crops of 1935-1939 and had been stored in a dark closet at room temperature. The honeys obtained this year were of the crops of 1940-1942. Many of these samples were received in the comb; others had been extracted before shipment. The difference in the pantothenic acid content of these two groups may be explained by the fact that this vitamin is unstable in an acid solution. Honey is naturally acid, having a pH range of 4-5. This acidity is not very great, but any

TABLE 2

Comparison of aged and new honeys.

All values are in micrograms per 100 gm.

		RIBO-FLAVIN	PANTO-THENIC ACID	NICOTINIC ACID	THIA-MINE	PYRI-DOXINE
Honeys of years						
1935-1939	Mean	21.8 ± 3.0	20.4 ± 2.4	124.4 ± 11.6	3.5 ± .25	7.6 ± .66
	Range	9 — 64	9 — 60	63 — 600	1.4 — 6.2	4 — 14
Honeys of years						
1940-1942	Mean	26.3 ± 2.1	54.4 ± 3.6	108.5 ± 9.1	4.4 ± .50	10.0 ± 1.1
	Range	7 — 60	20 — 360	72 — 590	2.2 — 12	4 — 27

pantothenic acid that might be in the honey could be destroyed slowly over a period of years. This is of little significance to the beekeeper or the housewife who do not keep honey for long periods of time.

A few honey samples were analyzed for their biotin and folic acid content, two new members of the B-complex whose importance in nutrition is being investigated. Traces of biotin and folic acid were found, with the average values of 0.066 µg. and 3 µg. per 100 gm., respectively. Since the amounts found were very small as compared to other foods, we did not examine many honeys.

Table 3 presents a comparison of the vitamin content of the samples of honey of the years 1940-1942 with pollen and royal jelly. It may be noted that the royal jelly, a milky-white sub-

stance secreted by the worker bee is unusually high in panto-
thenic acid and biotin. This may be related to the rapid meta-
bolism in the young bee larvae which depend on this substance
for sustenance the first 3 days after emergence from the egg.
Comparison of the vitamin content of pollen with that of honey
revealed that in general pollen was a hundred times richer in
the B vitamins than honey. This suggested that the vitamins
in the honey may be due partially to the pollen present in each
sample. All natural honeys contain a certain amount of pollen,
which at times is very small in quantity. Haydak and Palmer
('42a) have found that a commercial process of clarifying

TABLE 3

Comparison of honey with pollen and royal jelly.

All values are in micrograms per gram.

	NO OF SAMPLES	PANTO- THENIC ACID	RIBO- FLAVIN	NICO- TINIC ACID	THIA- MINE	PYRI DOXINE	BIOTIN	FOLIC ACID
Honey	19	0.55	0.26	1.1	0.044	0.10	0.00066	0.03
Pollen	2	27.0	16.7	100.0	6.0	9.0	0.25	..
Royal jelly	1	320 0	28.0	111.0	18.0	10.2	4.1	0.5

honey reduces the vitamin contents 33–50% of the original
values. When we filtered diluted honey, through Whatman
no. 40 filter paper subsequent to its preparation for assay, we
found that the riboflavin content was reduced 40% and the nico-
tinic acid values 25%. It is suggested that the large variation
in the vitamin content of the honeys may be due in part to the
variable amount of pollen present.

The values reported in this publication compare favorably
with those of other workers. Pearson ('42) reports an aver-
age value of 30.3 µg. of pantothenic acid per gram of pollen
and 511 µg. per gram (dry weight) of royal jelly. Cheldelin
and Williams ('42) have analyzed royal jelly for the vitamins
of the B complex. We find that our results agree very well
with those of the Texas workers. Haydak and Palmer ('42b)
report that chemical determinations did not show even traces
of pyridoxine either in bee bread or royal jelly. The values

they obtained for the amount of nicotinic acid and pyridoxine found in honey are many times higher than our results. The discrepancy is so great that it is believed to be due to the methods of analyses employed. It is significant to note that Haydak and Palmer used chemical methods for both the nicotinic acid and pyridoxine determinations. It is probable that the treatment of the honey sample necessary to free the vitamins gave rise to interfering substances.

SUMMARY

Microchemical and microbiological determinations showed the presence in honey of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, and folic acid. The variation among samples was very large, due perhaps to the source of the honey and the number of pollen grains present. Comparison of new and aged honeys revealed a decrease in the pantothenic acid content of the latter. Pollen and royal jelly have also been assayed for these vitamins and have been found to be good sources of the B-complex. Royal jelly is very rich in biotin and pantothenic acid, which may be significant in the metabolism of the young bee.

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THE DESTRUCTIVE ACTION, IN VIVO, OF DILUTE ACIDS AND ACID DRINKS AND BEVERAGES ON THE RATS' MOLAR TEETH

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ONE PLATE (EIGHT FIGURES)

A pronounced destruction of dentine and enamel of rats' molar teeth resulted from dilute acid solutions and certain acid drinks and beverages, consumed regularly in place of drinking water. In addition to effects observed both grossly and microscopically, differences in weights of comparable teeth of control and test rats gave a quantitative measure of actual amounts of dental tissue destroyed. The results point to the extreme sensitivity of rats' molar teeth, in vivo, to acid destruction, and suggest the possibility of a corrosive effect on human teeth resulting from excessive consumption of acid liquid-foods and beverages which may form a part of the human diet (Bridges and Mattice, '39). Acid foods such as ensilage, used extensively in animal nutrition, also may be suspected of having a corrosive action on the teeth.

In vitro studies have established acid solubility data for devitalized dentine and enamel (Rosebury, '38). Acid etching of enamel surfaces, in vitro, has been revealed by the reflecting microscope (Kanthak and Benedict, '32). Obviously results obtained in vitro do not constitute a measure of any acid-resistant properties of the live tooth, or of the acid-neutralizing effect of the saliva. Thus, although the destruction of tooth enamel by solutions of acidified candies was demonstrated in

vitro, it was suggested by West and Judy ('38) that the buffer action of the saliva would probably prevent any tissue destruction actually occurring in the mouth. Local oral effects resulting from consumption of certain foods have been studied largely as regards injury to the gingival tissues (Burwasser and Hill, '39; Pelzer, '40).

<div align="center">EXPERIMENTAL</div>

Part I

In this experiment weanling rats, divided into litter-mate trios, were allowed as the fluid to drink (a) distilled water, (b) a dilute solution of hydrochloric acid (pH 1.5, 0.16%), and (c) a dilute solution of lactic acid (pH 2.4, 0.50%). All the rats were fed a stock diet having the following percentage composition: corn flour, 66; whole milk powder, 30; alfalfa meal, 3; and salt, 1. Food and drinking fluids were consumed ad libitum. At the end of specified periods (table 1) the rats were sacrificed. After autoclaving the heads, the molar teeth were dissected out, dried at 110°C., and weighed.

Both lactic acid and hydrochloric acid were very destructive to tooth enamel and dentine (table 1, figs. 2 and 3). As might be expected hydrochloric acid pH 1.5 was more destructive than lactic acid pH 2.4. Observed microscopically, dissolution was particularly severe on the lingual surface of the lower molars. The upper molars, apparently because of more limited contact with the drinking fluids, were less severely affected. As shown in figures 2 and 3, the crowns of the lower teeth were almost entirely destroyed by these acids.

On the occlusal surface the dentine of the rats' molars is exposed naturally. In several instances a hollowed-out or cupped effect on the occlusal surface was observed, due apparently to the greater acid solubility of the dentine than of the enamel. In general, however, at these acid levels the enamel appeared to be affected at about the same rate as the dentine.

Differences in weights of individual molar teeth of control and test rats (table 1) indicate quantitatively actual weights of dental tissue destroyed. These weight figures, obtained

TABLE 1

Effect of dilute hydrochloric acid and dilute lactic acid as drinking fluids on rats' molar teeth.

DRINKING FLUID [1]	DAYS ON EXPERI-MENT	INITIAL BODY WT.	FINAL BODY WT.	WEIGHT OF MOLAR TEETH						LOSS IN WEIGHT	
				Right-lower			Right-upper			Right lower	Right upper
				1st	2nd	3rd	1st	2nd	3rd		
		gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	%	%
Water	42	44.1	114.5	10.6	6.5	4.3	12.1	6.9	3.0	...	..
Dilute HCl	42	33.7	96.0	8.3	5.4	2.6	10.9	6.4	2.7	23.8	9.1
Dilute lactic	42	36.2	109.5	9.1	5.7	3.0	11.5	6.5	2.8	16.8	5.5
Water	49	36.6	143.0	12.5	8.8	5.5	13.5	8.4	4.3	...	..
Dilute HCl	49	32.3	96.0	9.9	6.3	3.4	10.9	6.3	3.0	26.9	22.9
Dilute lactic	49	35.4	139.5	10.8	6.8	3.9	12.3	7.0	3.3	19.7	11.8
Water	56	27.0	107.5	11.5	7.9	5.1	12.5	7.9	3.8	...	..
Dilute HCl	56	24.8	109.5	7.3	4.3	2.5	8.3	4.7	2.2	42.4	37.2
Dilute lactic	56	27.2	116.5	8.7	5.7	3.4	10.2	5.8	2.4	27.3	23.9
Water	56	26.5	143.0	13.9	9.1	5.0	13.8	8.2	3.3	..	..
Dilute HCl	56	24.4	155.0	6.4	4.2	2.9	10.6	6.0	3.0	51.7	22.5
Dilute lactic	56	31.0	87.5	7.3	5.6	3.1	13.2	7.1	3.6	42.9	5.5
Water	56	36.0	130.0	12.9	8.6	5.0	13.8	7.1	3.3	...	.
Dilute HCl	56	25.2	136.4	8.8	5.2	2.7	12.5	5.8	2.5	36.9	14.0
Dilute lactic	56	23.4	162.5	8.0	4.7	3.1	12.4	7.5	3.3	40.4	4.1
Water	63	30.0	138.2	13.1	9.0	5.6	13.7	8.5	3.2	..	.
Dilute HCl	63	35.0	100.0	6.8	4.5	2.4	11.1	5.3	2.5	50.5	25.6
Dilute lactic	63	25.3	153.5	8.2	5.0	2.6	11.8	7.2	2.8	43.0	14.1
Water	63	23.4	125.4	12.0	8.2	4.8	12.1	7.5	3.2	...	..
Dilute HCl	63	29.2	130.0	9.1	6.0	3.2	10.9	5.8	2.7	26.8	14.9
Dilute lactic	63	23.3	108.0	11.5	6.9	3.5	12.0	7.4	2.7	12.4	3.1
Water	70	19.0	103.5	11.0	7.4	4.0	11.9	7.0	3.0	...	..
Dilute HCl	70	22.9	151.0	9.4	5.6	3.0	11.6	6.2	2.6	19.6	6.8
Dilute lactic	70	33.2	132.0	9.9	6.3	3.5	11.6	6.8	2.9	12.1	2.7
Water	70	24.6	133.0	12.3	8.0	4.8	12.8	7.9	3.8	.	..
Dilute HCl	70	25.5	152.0	8.6	5.1	2.2	10.5	5.8	2.5	36.7	23.3
Dilute lactic	70	21.8	118.5	10.9	6.9	4.0	13.0	7.2	3.6	13.1	2.9
Water	70	24.8	125.0	12.3	8.1	4.8	13.7	8.5	3.7	...	..
Dilute HCl	70	24.0	130.0	8.3	4.9	2.8	10.5	6.0	3.0	36.5	24.7
Dilute lactic	70	24.9	151.0	10.4	6.3	3.2	12.8	7.2	3.6	21.0	8.9
Water	70	23.2	116.5	13.1	8.5	4.8	13.4	7.8	3.5	...	..
Dilute HCl	70	21.6	128.0	10.2	6.5	2.7	12.4	6.7	2.8	26.5	11.3
Dilute lactic	70	22.2	121.5	10.5	6.7	3.3	12.9	7.6	3.5	22.3	2.8

Averages for each drinking fluid

Water	..	28.8	125.4	12.3	8.2	4.9	13.0	7.8	3.5	...	..
Dilute HCl	..	27.1	125.5	8.5	5.3	2.8	10.9	5.9	2.7	34.6	19.8
Dilute lactic	..	27.6	127.3	9.6	6.1	3.3	12.2	7.0	3.1	25.2	8.2

[1] Hydrochloric acid 0.16%, pH 1.5; lactic acid 0.50%, pH 2.4; distilled water pH 6.5–7.0.

under the condition of controlled fluid intakes, afford a quantitative means of comparing one acid fluid with another (table 2, experiment 3). Any suggestion of a difference related to the length of the exposure, among the rats shown in table 1, would have to be confirmed by equalizing the quantities of liquid drunk. Variations in results obviously may be brought about by differences in the rats' drinking habits and possibly by differences in individual susceptibilities.

TABLE 2

Weight of rats' molar teeth following consumption of a ginger ale, a cola drink, a grapefruit juice, and a cranberry juice cocktail as the only drinking fluids.

	WATER		GINGER ALE		COLA DRINK		GRAPEFRUIT JUICE		CRANBERRY JUICE COCKTAIL	
Drinking fluid reaction (pH)	6.5–6.6		2.8–3.2		2.6–2.7		3.2–3.5		2.5–2.6	
Drinking fluid total acidity (normality)	..		.06		.04		.22		.14	
Experiment number	2	3	2	3	2	3	2	3	2	3
No. of rats averaged	10	12	10	12	10	12	10	12	8	12
Days on experiment	82	84	82	82	82	82	82	85	80	82
Initial body weight (gm.)	33	42	27	41	33	42	30	40	30	44
Final body weight (gm.)	198	182	207	183	194	183	201	196	179	180
Average daily drink (ml.)	..	20.1	..	23.0	..	22.9	..	22.5	..	23.0
Average weight of molar teeth										
Right lower										
1st molars (mg.)	15.6	15.0	14.8	13.7	14.2	13.9	12.8	13.6	9.1	10.4
2nd molars (mg.)	9.9	9.9	9.2	9.0	8.9	9.0	7.9	9.1	5.8	7.2
3rd molars (mg.)	6.2	6.6	5.5	5.9	5.3	5.7	4.4	5.3	2.9	4.2
Right upper										
1st molars (mg.)	16.2	15.1	16.2	15.4	15.4	15.5	16.0	16.3	13.3	14.8
2nd molars (mg.)	9.7	9.4	9.6	9.5	9.3	9.4	9.7	10.3	7.5	9.3
3rd molars (mg.)	5.2	5.3	5.1	5.5	5.1	5.4	5.2	5.7	2.9	4.8

In addition to the results for lactic acid (pH 2.4) and hydrochloric acid (pH 1.5) it has been observed that citric acid (pH 2.5) (fig. 4) and lactic acid (pH 3.0 and 4.0) will corrode rats' molar teeth. The upper molars were not changed significantly.

Part II

The above study was followed by experiments 2 and 3 (table 2) in which the following liquids were drunk by young rats in place of water: (a) ginger ale pH 2.8–3.2; (b) a cola drink pH 2.6–2.7; (c) grapefruit juice pH 3.2–3.5 and (d) a cranberry juice cocktail pH 2.6–2.7. These drinks and beverages were obtained from local food stores. The ration was a commercial one.[1] In experiment 2 eating and drinking were allowed ad libitum, and in experiment 3, with the exception of water, fluid consumption was equalized. Fresh allotments of drinking fluid were given each day. These beverages did not change in pH after standing 24 hours at room temperature.

The weights of the molar teeth of these rats are shown in table 2. Differences between control and test rats are most pronounced in the lower molar teeth. These weight differences minimize the corrosive action because a large portion of the weight of the tooth consists of the unexposed root and it would appear also that in general the buccal surfaces of the teeth have relatively little contact with the drinking fluids. During the drinking process the lower molars seemingly are in contact with the drinking fluids more than the uppers. This is shown by the weight data and by the appearance of the teeth. As observed microscopically, all the rats given acid fluids showed an unmistakable corrosion on the lower molars in particular. The photographs (figs. 5, 6, 7 and 8) also show this effect. In the rats given cranberry juice cocktail the crown of the lower molars was frequently reduced to the gingival line. The most serious action resulted from this fluid. In experiment 3 where the fluid intakes were controlled, the other three fluids were similar in their corrosive action. There was some tendency for the upper molars of control rats to weigh slightly less than the upper molars of test rats. This may be explained possibly by a greater loss from normal occlusal wear in these rats than in the test rats. As a result of the acid fluids the lower molars of test rats frequently showed a more or less

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[1] Stock rat ration, a product of the Maritime Milling Co., Buffalo, New York.

smooth diagonal surface which presumably lessened the normal wear on the opposing upper molars.

An estimate of the pH of the rats' oral cavities was obtained by means of a nasal electrode placed in the mouths of rats while they were under the influence of a mild ether anesthetic. These readings on eight rats equaled pH 8.2, 8.2, 8.0, 7.7, 8.0, 7.8, 7.9, and 8.0. Several 1 ml. volumes of washings from rats' oral cavities averaged pH 7.8 to pH 8.0. The possibility that a reduced buffering action of rat saliva contributed to these acid effects seems negligible.

DISCUSSION

A current conception of the etiology of dental caries is the decalcification of the dental tissues by acids of bacterial origin particularly lactic acid (Miller, 1890). The acid resistant properties of teeth would appear to play an important rôle in the prognosis of the disease. These results pertaining to rats' teeth in vivo suggest the possibility that oral tooth surfaces may be affected by acid drinking fluids passing through the oral cavity. Any association of these effects with the initiation of dental caries however, remains to be determined. The experimental procedure permits the observation in vivo of the susceptibility of dental tissues to various acids of dietary or bacterial origin, and has the additional advantage of furnishing quantitative data relative to the actual quantities of dental tissue destroyed.

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Enlarged photographs showing corrosive effect of dilute acids and acid drinks and beverages on the rats' molar teeth: (1) control, distilled water pH 6.5–7.0; (2) hydrochloric acid pH 1.5; (3) lactic acid pH 2.4; (4) citric acid pH 2.5; (5) a cola drink pH 2.6–2.7; (6) a ginger ale pH 3.2–3.4; (7) grapefruit juice pH 3.2–3.5; (8) a cranberry juice cocktail pH 2.5–2.6.
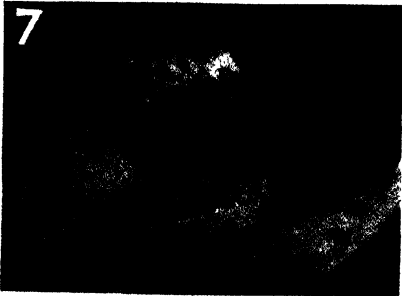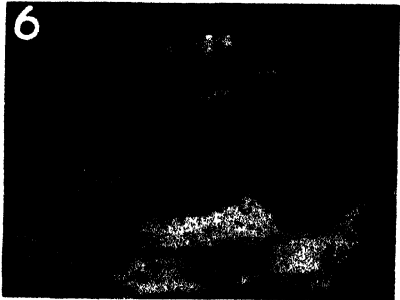
THE INFLUENCE OF THE THIAMINE INTAKE OF THE PIG ON THE THIAMINE CONTENT OF PORK WITH OBSERVATIONS ON THE RIBOFLAVIN CONTENT OF PORK [1]

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It is well-established that pork is an outstanding source of thiamine. A survey of the literature reveals, however, that there is considerable variation in the thiamine values which have been reported for pork. While some of these differences might be attributed to methods of determination, and to normal or expected variation, it is logical to suppose that certain physiological factors might also be responsible, at least in part, for some of the diversity among reported thiamine values for pork.

Among such factors are thiamine saturation of tissues (Schultz, Light and Frey, '38; Schultz, Light, Cracas and Atkin, '39), the relationship of thiamine to the glycogen cycle in muscle (Pyke, '40), and the thiamine sparing action of fats (Ellis and Madsen, '42).

It is a generally accepted fact that increased intake of carbohydrate increases the thiamine requirement; Waisman and Elvehjem ('41) have commented on this fact in connection with swine which are fed rations characteristically rich in

carbohydrate. It is possible that the characteristically high thiamine content of pork is the result of demands on the muscle tissue for thiamine to aid in the metabolism of carbohydrate, including its conversion to fat.

A further possibility is that the thiamine content of pork might be directly influenced by the thiamine intake of the pig. Some data bearing on this point have been presented by Pyke ('40) who reported thiamine values for the longissimus dorsi and psoas (back and loin) muscles from groups of two, five and four pigs fed, respectively, on swill, commercial meal (190 I. U. thiamine per 100 gm.), and the same commercial meal supplemented with crystalline thiamine to raise its thiamine content to 850 I. U. per 100 gm. No assay values are reported for the swill, but it is stated that it almost certainly contained less vitamin B_1 than the cereal meal. The amount of thiamine in the psoas muscle was shown to be consistently greater than that present in the longissimus dorsi. The thiamine values for these muscles were very similar for the pigs fed the plain meal and for those fed the meal supplemented with thiamine, but the values were considerably higher, in each instance, than those for the two swill-fed pigs. Pyke concludes that a normal pig ration supplies all the vitamin B_1 that the pig is capable of storing, and that the reduction in muscle thiamine in the swill-fed pigs is due to a deficiency of thiamine in the diet.

The present investigation was undertaken to obtain further data dealing with the relationship between the thiamine intake of the pig and the thiamine content of the pork. Although the study was primarily concerned with thiamine, riboflavin values were also determined owing to the paucity of riboflavin values reported in the literature.

EXPERIMENTAL

The feeding experiment was conducted during the period July to November, 1942. Twenty-seven pigs were assigned to three lots of nine pigs each, with the distribution between lots being made as nearly equal as was possible with respect

to breed, sex and initial live weight (table 2). Throughout most of the feeding period the pigs were kept on the ground in half-acre outdoor feed lots which were devoid of vegetation. Suitable shelter against the sun and inclement weather was provided, and in the latter part of the experiment, in colder weather, the pigs were housed indoors.

The pigs were trough-fed in groups, twice daily. Data on feed consumption were recorded and no more feed was offered than could be readily consumed. A suitable amount of water was added to the feed at feeding time.

TABLE 1

Composition of rations.

| | INGREDIENTS | | |
	Lot 1	Lot 2	Lot 3
	%	%	%
Yellow corn	57.0	49.0	53.5
Tankage (60% protein)	5.5	5.5	11.0
Soybean oil meal	10.0	10.0	——
Linseed oil meal	10.0	10.0	——
Dehydrated alfalfa leaf meal	5.0	5.0	10.0
Whole wheat	——	10.0	15.0
Peanut skins	10.0	10.0	——
Corn gluten meal	——	——	10.0
Dried brewers' yeast	2.0	——	——
Salt	0.48	0.48	0.48
Manganese	0.02	0.02	0.02
	100.00	100.00	100.00
	ANALYSIS		
Protein (%)	19.95	19.35	19.78
Thiamine (μg. per lb.)	5761	3447	1315
Riboflavin (μg. per lb.)	1801	1669	2468

The composition of the rations is given in table 1. The protein content of the rations is considered adequate for pigs weighing 40 to 50 pounds, but it is higher than that required for pigs weighing from 75 to 200 pounds. Rations 1, 2 and 3, fed to lots 1, 2 and 3, respectively, were designed primarily to furnish three levels of thiamine, the respective amounts being 5761, 3447 and 1315 µg. of thiamine per pound of feed. Ex-

traordinary sources of thiamine were red peanut skins, used
in rations 1 and 2, and brewers' yeast, used in ration 1. Some
difficulty was experienced in obtaining brewers' yeast which
contained sufficient thiamine to provide the desired amount
in ration 1. Consequently, the required additions of pure thia-
mine (in solution) were mixed with this ration just prior to
feeding.

Three pigs, one from each lot, were slaughtered on the
100th day of the feeding experiment. Thereafter, usually at
weekly intervals, one pig from each lot was slaughtered.
Thus the length of the feeding period varied from 99 to 140
days, respectively, for the first and last pigs slaughtered from
each lot. After slaughter the carcasses were chilled for at
least 24 hours at 37°F. before samples of the pork were taken
for analysis. Samples were taken from the ham end of the
loin, the center of the loin, the shoulder and the liver. Ad-
hering fat was removed from the samples which were pre-
pared for analysis by grinding in a food chopper. Thus,
all analyses represent lean tissue.

Whenever possible, samples for the determination of dry
matter, crude protein and thiamine were weighed immediately
after the sample had been ground and thoroughly mixed.
Otherwise samples were frozen immediately in the freezing
compartment of an electric refrigerator and kept in the frozen
state until they were analyzed. All of the riboflavin deter-
minations were made on samples which had been kept in the
frozen state.

Thiamine was determined by the thiochrome method, us-
ing the procedure recommended for cereal products (Hen-
nessy, '42), and riboflavin was determined by the fluorometric
method of Conner and Straub ('41). These methods were
modified only in the manner of preparation of the extracts;
a weighed amount of the previously ground fresh or frozen
sample was mixed in a Waring blender with a suitable volume
of extractant, from which mixture aliquots were pipetted into
volumetric flasks.

TABLE 2

Weights, feed consumption and economy of gains of pigs with different levels of thiamine intake.

LOT NO.	THIAMINE CONTENT OF FEED	PIG NO.	BREED	SEX	AGE AT START OF EXPERIMENT	NUMBER OF DAYS ON FEED	INITIAL LIVE WEIGHT	FINAL LIVE WEIGHT	TOTAL GAIN IN LIVE WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY FEED	FEED REQUIRED PER 100 LBS. GAIN IN LIVE WEIGHT
	μg/lbs.				days		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	5761	988	Chester White	Barrow	88	99	55	209	154	1.56	4.7	301
		990	Poland China	Barrow	74	105	49	207	158	1.50	4.8	319
		996	Chester White	Barrow	88	111	56	205	149	1.34	5.0	372
		995	Duroc Jersey	Gilt	74	112	44	200	156	1.39	5.0	361
		65	Duroc Jersey	Gilt	74	119	50	205	155	1.30	5.2	395
		70	Berkshire	Gilt	77	127	44	205	161	1.27	5.3	329
		74	Chester White	Gilt	88	133	34	205	171	1.29	5.4	422
		916	Poland China	Barrow	74	140	49	197	148	1.06	5.5	522
				Average	80	118	48	204	157	1.34	5.1	378
2	3447	992	Chester White	Gilt	88	99	61	226	165	1.67	4.9	297
		991	Chester White	Barrow	88	105	56	211	155	1.48	5.0	340
		998	Chester White	Barrow	88	111	49	200	151	1.36	5.2	382
		997	Duroc Jersey	Gilt	74	112	50	210	160	1.43	5.2	365
		66	Duroc Jersey	Barrow	74	119	47	220	173	1.45	5.2	278
		71	Poland China	Barrow	74	127	41	222	175	1.38	5.4	390
		75	Berkshire	Gilt	82	133	35	202	167	1.26	5.5	435
		917	Berkshire	Barrow	102	140	31	167	136	0.91	5.4	560
				Average	84	118	46	207	160	1.37	5.2	381
3	1315	994	Chester White	Barrow	88	99	52	212	160	1.62	5.0	308
		993	Duroc Jersey	Gilt	74	105	50	200	150	1.43	5.1	354
		999	Duroc Jersey	Barrow	74	111	45	200	155	1.40	5.2	375
		1000	Duroc Jersey	Gilt	74	112	47	218	171	1.53	5.3	345
		68	Chester White	Gilt	88	119	47	210	163	1.37	5.3	260
		73	Berkshire	Barrow	82	127	45	185	140	1.10	5.4	492
		915	Chester White	Gilt	88	133	49	198	149	1.12	5.5	494
		918	Poland China	Barrow	74	140	40	210	170	1.21	5.7	466
				Average	80	118	47	204	157	1.35	5.3	387

EXPERIMENTAL RESULTS AND DISCUSSION

Data pertaining to the body gains and feed consumption of the pigs are given in table 2. It is shown that, on the average, in spite of individual differences, the three lots of pigs were quite similar with respect to growth and feed requirements. It is apparent that the ration for lot 3, which furnished the least amount of thiamine was fully adequate to fulfill the pigs' requirements for this factor. In this case the average daily feed intake of 5.3 lbs. supplied 7 mg. of thiamine which is in

TABLE 3

Average proximate analyses of pork.

LOT NO		1	2	3
NUMBER OF PIGS		8	8	8
THIAMINE CONTENT OF FEED (μG./LB.)		5761	3417	1315
SOURCE OF SAMPLE		%	%	%
Shoulder	Dry matter	27.0	28.5	28.2
	Protein in fresh pork	19.2	19.2	19.5
Center loin	Dry matter	27.8	27.6	29.2
	Protein in fresh pork	21.7	21.8	21.8
Ham end of loin	Dry matter	26.9	28.0	28.4
	Protein in fresh pork	21.4	21.8	21.2
Liver	Dry matter	30.5	31.0	30.5
	Protein in fresh liver	23.1	22.5	22.2

excess of the suggested daily requirement of 1 to 3 mg. per 100 lbs. of body weight. The results show, further, that there was no apparent advantage derived from the additional thiamine provided in the rations of lots 1 and 2 so far as growth and feed utilization are concerned.

The average proximate analyses of the pork are given in table 3. Again, in spite of individual differences, there is general uniformity of the results for the pigs of the three lots.

Data for the thiamine content of the pork are given in table 4. Thiamine values for the muscle tissues directly reflect

TABLE 4

The thiamine content of pork as influenced by the thiamine content of the feed (thiamine expressed as μg./gm. of fresh tissue, dry matter and protein).

PIG NO.	SHOULDER			CENTER LOIN			HAM END OF LOIN			LIVER		
	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein
				Lot 1 — 5761 μg. of thiamine per pound of feed								
988	16.0	51.7	87.7	20.1	61.8	99.0	22.3	80.2	106.8	5.9	19.3	24.2
990	13.1	44.2	65.7	18.3	69.9	81.3	17.8	65.5	80.9	2.7	9.5	12.2
996	19.4	76.6	95.2	25.1	86.1	110.9	26.3	93.5	120.9	7.3	24.5	31.8
995	17.9	63.8	96.1	25.3	87.8	112.3	24.4	93.9	120.5	6.2	20.7	26.7
65	14.7	59.2	80.3	23.3	86.5	109.6	22.6	87.8	110.2	6.2	19.9	27.5
70	17.2	69.5	85.4	23.8	89.5	106.1	21.8	81.7	97.7	3.6	11.9	16.2
74	19.2	71.2	102.1	22.9	79.6	105.4	25.8	90.9	123.6	5.6	17.3	24.5
916	21.1	82.5	109.2	27.8	106.9	125.3	29.9	117.0	132.5	5.2	16.6	21.4
Average	17.3	64.8	90.2	23.1	83.5	106.2	23.9	88.8	111.6	5.3	17.5	23.1
Standard error	0.9	4.5	4.8	1.0	4.8	4.4	1.3	5.2	5.6	0.5	1.7	4.8
				Lot 2 — 3447 μg. of thiamine per pound of feed								
992	13.1	41.8	69.4	19.9	69.6	90.5	18.6	63.2	85.3	4.8	15.5	20.1
991	16.8	57.7	86.7	23.4	80.9	106.7	24.2	86.8	108.8	4.8	15.7	20.4
998	15.5	51.1	78.5	18.3	65.3	82.2	19.6	67.1	85.4	4.4	15.3	21.9
997	16.9	57.5	87.5	20.7	77.8	92.0	19.4	70.3	93.5	5.1	15.9	21.5
66	12.6	49.6	67.0	20.1	77.1	94.6	19.3	76.0	91.3	5.5	18.0	23.0
71	14.0	46.2	75.4	18.8	65.8	90.9	19.2	64.2	89.8	4.2	14.2	18.9
75	13.8	52.7	69.0	19.0	69.0	84.0	19.9	72.9	88.2	3.9	11.8	18.1
917	17.8	68.3	92.5	20.1	76.7	93.8	20.5	75.8	95.3	3.0	9.3	14.2
Average	15.1	53.1	78.3	20.0	72.9	91.8	20.1	72.0	92.2	4.5	14.5	19.8
Standard error	0.7	2.9	2.9	0.6	2.1	2.6	0.6	2.7	2.7	0.3	1.0	1.0
				Lot 3 — 1315 μg. of thiamine per pound of feed								
994	6.5	20.1	35.6	9.2	28.7	44.3	10.4	37.3	52.0	4.2	13.9	18.6
993	5.9	23.5	31.0	7.3	24.5	32.7	11.1	38.5	52.1	3.8	11.9	15.1
999	10.1	37.4	49.0	9.6	30.9	43.1	10.0	33.1	48.3	3.6	10.7	16.6
1000	7.7	25.1	41.3	9.2	31.5	45.3	8.8	28.6	44.3	3.5	11.5	15.2
68	7.1	28.0	33.9	10.5	40.4	45.7	9.5	36.4	42.2	2.5	8.2	12.1
73	9.7	35.2	46.6	10.4	36.7	45.8	12.1	43.4	52.6	1.9	6.4	8.2
915	7.5	26.4	39.1	9.7	35.9	44.0	9.5	33.8	43.3	2.4	8.2	10.5
918	8.9	30.6	47.3	10.3	33.9	49.9	11.2	41.3	55.1	4.5	15.5	24.1
Average	7.9	28.3	40.5	9.5	32.8	43.9	10.3	36.6	48.7	3.3	10.8	15.1
Standard error	0.5	2.1	3.1	0.4	1.9	1.8	0.4	1.7	1.7	0.3	1.1	1.8

the thiamine intakes of the pigs, and the data show that much higher amounts of thiamine were present in the muscle tissues of the pigs of lots 1 and 2 than was the case for similar tissues of the pigs of lot 3. The thiamine content of the pork from the pigs of lot 2, which received 3447 μg. of thiamine per pound of feed, is approximately twice that of the pigs of lot 3, which received 1315 μg. of thiamine per pound of feed. In the case of lot 1, which received 5761 μg. of thiamine per pound of feed, the average thiamine values for the pork are higher, in all instances, than those for the pork from the next lower thiamine lot (no. 2), but, although the trend of the values seems to be established, the differences are not statistically significant in all cases. The above relationships are true whether or not the comparisons are made on the basis of the thiamine content of the fresh pork, dry matter, or total protein. Although the thiamine values for the pork from lot 1 are higher than those for lot 2, the differences are not commensurate with the differences in the thiamine intake of these two lots. A more direct relationship exists in the case of lots 2 and 3 where an increase of 2.6 times in thiamine intake resulted in approximately doubling the thiamine content of the pork.

It is interesting to note that there seems to be a preferential attraction of pork muscle for thiamine as compared to liver. Furthermore, liver seems to be much less responsive to the effect of added dietary thiamine. It will be noted, in comparing lots 2 and 3, that the increase in thiamine content of liver, as the result of thiamine feeding, was of the order of 40 to 70%, as contrasted with about 100% for muscle. Pork muscle is unique in its ability to absorb and retain thiamine and appears to be the principal repository of this vitamin.

Our findings with respect to the increased thiamine content of pork muscle as the result of feeding are somewhat similar to those reported by Pyke ('40), but they differ in several respects the first of which is the magnitude of the thiamine values for pork from pigs with more or less comparable intakes of thiamine.

The limited data of Pyke show a range of from 12 to 14.55 µg. of thiamine per gram of loin on feeds which ranged from 2586 to 11,567 µg. of thiamine per pound of feed. Our thiamine values are somewhat higher, ranging from 18.3 to 27.8 µg. per gram of loin from pigs which received 3447 and 5761 µg. of thiamine per pound of feed. In spite of the difference in the magnitude of the thiamine values, our observations and those of Pyke show that different muscle tissues from the same pig may contain different amounts of thiamine. In our studies the loin values exceeded those of the shoulder by 20 to 25%. No data are available showing whether there may be a variation in the thiamine content of different parts of the same muscle.

Pyke's results show little, if any, increase in the thiamine values for pork when the level of thiamine feeding was increased from 2586 to 11,567 µg. per pound of feed, although the values at these levels were approximately double those for swill-fed hogs. Our values for pigs fed 3447 µg. of thiamine per pound of feed are approximately 30% higher than those reported by Pyke at the highest level of feeding (11,567 µg. per pound of feed), and in each instance our values show some increase as a result of a higher level of thiamine intake.

There does not appear to be any satisfactory explanation for the above points of difference. Feed consumption might be a factor influencing thiamine storage. Pyke does not report the feed intakes, but in our case it was practically the same for the three lots of pigs. Our results do not indicate any marked influence of breed on the thiamine content of the pork. However, Pyke's data indicate that breed might be a factor, and it is possible that the Wessex Saddleback pig might differ in this respect from the breeds used in our investigation. We feel, however, that the species rather than the breed, in the case of swine, characterizes the great ability of the pig to accumulate thiamine in its muscle tissue.

Our results, which show that the thiamine content of pork may be influenced by the thiamine intake of the pig, and that different pork cuts may vary in thiamine content, are of

particular interest in helping to explain some of the wide variations in thiamine values for pork which have been reported in the literature. Our work does not fully delineate the limits within which the thiamine content of pork may vary, but some indication of the upper limit is indicated by the results obtained by feeding 5761 µg. of thiamine per pound of feed to lot 1, and by further data given in table 5.

TABLE 5

Thiamine and riboflavin values for pork from pigs receiving 50 mg. of additional thiamine hydrochloride daily for a period of 22 days

PIG. NO		69 [1]	72 [2]	69	72
SOURCE OF SAMPLE	BASIS	THIAMINE		RIBOFLAVIN	
		µg /gm.	µg /gm.	µg /gm.	µg /gm.
Shoulder	Fresh	15.5	14.1	3.5	3.7
	Dry matter	59.8	50.1	13.5	13.1
	Protein	79.2	71.6	17.9	18.7
Center loin	Fresh	22.2	18.6	1.8	2.4
	Dry matter	89.2	66.2	7.4	8.4
	Protein	101.5	86.3	8.4	10.9
Ham end of loin	Fresh	21.8	18.1	2.9	3.3
	Dry matter	85.7	66.3	11.3	12.0
	Protein	102.3	84.7	13.4	15.3
Liver	Fresh	5.6	5.8	51.7	46.9
	Dry matter	19.0	17.0	175.3	137.5
	Protein	26.0	23.8	239.8	192.4

[1] Pig no. 69 received feed containing 5761 µg. thiamine per pound.

[2] Pig no. 72 received feed containing 1315 µg. thiamine per pound.

One pig from the original lot 2 was affected with piles and was removed from the experiment. Its mates in lots 1 and 3, nos. 69 and 72, were continued on the experiment and received the same feeds as the other members of lots 1 and 3, with the addition, during the last 22 days of the experiment, of a daily supplement of 50 mg. of pure thiamine. The results, as given in table 5, show that there was no accumulation of this additional thiamine in the muscle tissue of the pig (no. 69) on the high thiamine (5761 µg. per pound) feed; however,

a very appreciable storage of thiamine did occur in the case of pig no. 72, which had received only 1315 µg. of thiamine per pound of feed. The thiamine values for the pork of this pig are of the same order as those for the pork of the pigs of lots 1 and 2, and indicate that thiamine may be very rapidly accumulated in pork muscle tissue under conditions of high thiamine intake.

Schultz, Light, Cracas and Atkin ('39) have pointed out that storage of thiamine in the accepted sense, does not take place in the body of the rat. We feel that probably this is also true in the case of the pig. This viewpoint may involve a very strict differentiation between storage and accumulation. Since our limited data indicate that thiamine may be accumulated rapidly in pork muscle, under conditions of high thiamine intake, it is felt that its disappearance from the tissue may be equally rapid. Thus, thiamine accumulation in the tissue, even to the point of saturation, may not constitute true storage.

The data presented in this paper do not give a complete picture with respect to the average values and the lower limit of the thiamine values which may apply to pork in general and which may be determined by the thiamine intake of the average hog. Limited data are available for the thiamine content of hog feeds. It may be assumed, however, that many hog rations, especially those in which corn predominates heavily, are not rich in thiamine. On the other hand, hogs fed peanuts, peanut by-products, certain oil meals, yeast and other thiamine-rich feed ingredients ingest considerable amounts of thiamine. With the lack of any specific data to prove the point, we feel that 1315 µg. of thiamine per pound of feed, the amount present in the ration of lot 3, represents a thiamine intake for hogs which is probably somewhat higher than the average. Thus, the thiamine values for the pork of lot 3 are probably at least not lower than the average.

Average riboflavin values for pork from lots 1, 2 and 3 are given in table 6. No attempt was made to control the ribo-flavin content of the feeds. Consequently, the variations in this vitamin in the feeds, were not comparable to those of

thiamine. It will be noted that the riboflavin content of pork muscle is much lower than that of thiamine while pork liver is rich in riboflavin but relatively low in thiamine.

TABLE 6

The riboflavin content of pork.

LOT NO.		1		2		8	
NUMBER OF PIGS		8		8		8	
RIBOFLAVIN CONTENT OF FEED (µG /LB.)		1801		1669		2468	
SOURCE OF SAMPLE	BASIS	AVERAGE RIBOFLAVIN CONTENT OF PORK (EXPRESSED AS µG./GM. OF FRESH TISSUE. DRY MATTER AND PROTEIN)					
		µg./gm.	Standard error	µg./gm.	Standard error	µg./gm.	Standard error
Shoulder	Fresh	3.5	0.1	3.3	0.1	3.2	0.1
	Dry matter	13.0	0.7	11.5	0.5	11.5	0.9
	Protein	18.2	0.6	17.0	0.7	16.4	0.7
Center loin	Fresh	2.2	0.1	2.3	0.1	2.2	0.1
	Dry matter	7.9	0.4	8.1	0.4	7.5	0.4
	Protein	10.1	0.4	10.3	0.6	10.1	0.3
Ham end of loin	Fresh	3.2	0.2	3.0	0.2	3.0	0.2
	Dry matter	11.8	0.7	10.6	0.5	10.7	0.6
	Protein	14.9	1.0	13.6	0.6	14.3	0.7
Liver	Fresh	43.8	0.9	41.8	0.8	40.5	1.0
	Dry matter	143.7	3.0	134.9	2.3	132.7	2.7
	Protein	189.6	2.9	185.9	3.0	182.5	3.5

SUMMARY

Three lots of pigs were fed rations containing 5716, 3447 and 1318 µg. of thiamine per pound of feed, respectively. The average feed consumption was approximately the same for the three lots of pigs. Thus, the average daily thiamine intakes were approximately 29, 17 and 7 mg., respectively.

Results with respect to growth and feed consumption were similar for the three lots of pigs in spite of the differences in thiamine intake.

Thiamine values were determined on pork samples taken from the shoulder, the middle of the loin, the ham end of the

loin and the liver. An increase of approximately 100% in the thiamine content of pork muscle resulted when the thiamine intake was increased from 1318 to 3447 µg. per pound of feed. A similar increase of the order of 15 to 20% resulted from an increase in the thiamine content of the feed from 3447 to 5761 µg. per pound.

The average thiamine values, on the fresh basis, for shoulder, center loin and ham end of loin, at the lower level of thiamine intake, were 7.9, 9.5 and 10.3 µg. per gram, respectively. At the high level of thiamine intake the corresponding values were 17.3, 23.1 and 23.9 µg. per gram.

The thiamine content of pork loin was found to be consistently higher than that of pork shoulder. Pork liver, as compared to muscle, was found to be relatively low in thiamine content. There was some increase in the thiamine content of the liver as the result of feeding more thiamine, but the main depository of thiamine in the pig is the muscle tissue.

Limited observations with but two pigs seem to indicate that pork muscle tissues have nearly reached the saturation point, so far as thiamine storage is concerned, when the thiamine content of the ration approximates 5800 µg. per pound. As the result of these preliminary observations it is concluded that thiamine accumulation in pork muscle is relatively rapid. These experiments are being continued.

Riboflavin values were determined for all of the pork samples. The riboflavin content of pork muscle is low as compared to this tissue's content of thiamine. Pork liver is relatively high in riboflavin content.

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DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

XXXI. FURTHER STUDIES ON THE ROLE OF P-AMINOBENZOIC ACID AND INOSITOL IN LACTATION AND GROWTH OF THE ALBINO RAT [1]

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In 1941 it was shown that as a source of the vitamin B complex, adequate daily doses of pure crystalline thiamine, riboflavin, pyridoxine, choline chloride, calcium pantothemate and the "W" factor from liver extracts resulted in complete failure in lactation of the albino rat. The missing factor, tentatively designated as "Bx", was found in rice polishings, defatted wheat embryo, dried grass, and brewers' yeast, but most abundantly in liver and rice bran extracts (Sure, '41).

Following the suggestion of Ansbacher ('41) that p-aminobenzoic acid may be a component of the vitamin B complex, experiments were carried out to determine if this aromatic acid is a constituent of or identical with the "Bx" factor. Definite responses to p-aminobenzoic acid were obtained in lactation (Sure, '41); these have been now confirmed with numerous additional experiments, the results of which are presented in this communication.

The report of Woolley ('41) that inositol is an essential factor for growth of the mouse, as well as the claim of Pavcek and Baum ('41) that inositol is a growth-promoting substance for the rat, warranted its trial in lactation. However, the pre-

liminary positive responses in the case of only a few litters of nursing young were not confirmed by much more extensive studies in which inositol was used throughout the growth, reproduction, and lactation periods. The results of this investigation are presented in tables 1 and 2.

TABLE 1

Composition of Sure's salts no. 2.

	gm.		gm.
NaCl	335.0	$MnSO_4$ (anhydrous)	8.0
K_2HPO_4 (anhydrous)	645.0	$ZnCl_2$	0.5
$CaHPO_4.2H_2O$	190.0	$CuSO_4$ (anhydrous)	0.4
$MgSO_4$ (anhydrous)	99.0	$Al_2(SO_4)_3K_2SO_4$	0.4
$CaCO_3$	600.0	NaF	0.5
Ferric citrate (powdered)	55.0	Cobalt chloride	0.5
KI	1.6	Sodium tetra borate (anhydrous) $(Na_2B_4O_7)$	0.5

Before the new series of lactation experiments were initiated, a modification in the salt mixture was made, increasing the $MnSO_4$ from 7.0 to 8.0 gm., and adding cobalt and boron as trace elements, as a factor of safety, to insure optimum mineral requirements, although boron has not yet been shown to be essential for the animal organism. The composition of the new salt mixture, designated as Sure's salts no. 2, is given in table 1. To further insure adequate mineral intake, the new salt mixture was fed at a 5 instead of a 4% level. Because of the high moisture content and the lack of absolute purity, the blood fibrin rations were fed at a 25% plane of intake, which was the equivalent of 20% protein, calculated from the total nitrogen content.

It was realized that the "Bx" factor may be a composite of several unknown components of the vitamin B complex. Therefore, in order to secure a response to a single chemical substance such as p-aminobenzoic acid, small amounts of natural foods or extracts therefrom, were incorporated in some of the rations, i.e., 2% dried grass in ration 13, 2% dried liver in ration 14, and 1% liver concentrate in ration 12. Rations 12, 13, and 14 also contained 0.1% ascorbic acid. Numerous other

TABLE 2

The role of p-aminobenzoic acid and inositol in lactation.

RATION NO.	GROUP NO.	SOURCE OF PROTEIN	SOURCE OF CARBO-HYDRATES	SPECIAL ADDITIONS	p-AMINO-BENZOIC ACID OR INOSITOL ADDITIONS	NO OF FEMALES MATED	NO. OF LITTERS	TOTAL YOUNG BORN	YOUNG GIVEN TO BEAR	YOUNG REARED	PER CENT YOUNG REARED
13	1	{ Casein, 10% { Fibrin, 10%	Dextrin	Dried grass, 2%	Control	5	6	64	36	6	16.6
13		,,	Dextrin	,,	inos.[1,2]	5	6	56	35	0	0.0
13		,,	Dextrin	,,	p.a.b.-inos.[1,2]	5	8	65	41	16	39.0
12	2	{ Casein, 10% { Fibrin, 10%	Dextrin	Liver concentrate, 1%	Control	5	10	127	60	20	33.3
12		,,	Dextrin	,,	inos.[1]	5	11	96	63	10	15.9
12		,,	Dextrin	,,	p.a.b.-inos.[1]	5	9	106	54	33	61.0
14	3	{ Casein, 10% { Fibrin, 10%	Dextrin	Dried liver, 2%	Control	5	9	92	40	23	57.5
14		,,	Dextrin	,,	inos.[1]	5	9	88	53	5	9.4
14		,,	Dextrin	,,	p.a.b.-inos.[1]	5	11	109	60	51	85.0
8	4	Fibrin 25%	Dextrin	Yeast nucleic acid, 0.2%	Control	5	10	97	59	18	30.5
9		Fibrin, 25%	Dextrin	,,	inos., 0.2%	5	6	44	23	5	21.7
10		Fibrin, 25%	Dextrin	,,	p.a.b., 0.2%	5	6	67	36	18	50.0
11		Fibrin, 25%	Dextrin	,,	{ p.a.b., 0.2% { inos., 0.2%	5	6	50	16	5	31.2
7	5	Casein, 22.5%	Cerelose		Control	10	13	117	77	17	22.1
2	5	Fibrin, 25%	Cerelose		Control	15	32	340	216	87	40.3
3		Fibrin, 25%	Cerelose	Polished rice, 30%		5	11	96	66	44	66.6
4		Fibrin, 25%	Cerelose	,,	inos., 0.2%	5	12	112	67	21	31.3
5		Fibrin, 25%	Cerelose	,,	p.a.b., 0.2%	5	11	112	63	62	98.4
6		Fibrin, 25%	Cerelose	,,	{ inos., 0.2% { p.a.b., 0.2%	5	10	108	60	53	88.3
1		Fibrin, 25%	Dextrin	,,	Control	15	21	195	119	33	27.7

[1] Administered separately from the ration in doses outlined in the text.
[2] p.a.b. = p-aminobenzoic acid; inos. = inositol.

experiments, not included here, showed no special benefits in lactation from ascorbic acid additions. All the rations contained small amounts of nicotinic acid. The cystine additions to rations containing casein were made only during the reproduction and lactation periods. Two sources of carbohydrates were used, a commercial dextrose [2], and dextrin, the latter prepared in the laboratory by cooking cornstarch for 3 to 4 hours at 15 to 17 pounds pressure.

Adequate amounts of the various components of the vitamin B complex were administered separately from the rations in doses outlined in a previous publication (Sure, '41). Vitamins A and D were furnished by 3 drops of halibut liver oil to each animal once weekly until mating, and 3 drops three times weekly during pregnancy and lactation. Vitamin E was provided by wheat germ oil in the rations.

COMPOSITION OF RATIONS

The percentage compositions of diets 1 and 2 were as follows: blood fibrin [3] 25, butterfat 10, carbohydrate 56.95, Sure's salts (no. 2)[4] 5, wheat germ oil 3, and nicotinic acid 0.05. These diets differed merely in that diet 1 had dextrin as the carbohydrate whereas diet 2 had dextrose.[5] Diet 3 was the same as diet 2 except that it contained 30% polished rice with the dextrose reduced accordingly. Diet 4 differed from diet 3 in that the former had 0.2% inositol with a further corresponding reduction in the dextrose. Diet 5 was the same as diet 4 except that no inositol was present but, instead, 0.1% p-aminobenzoic acid; and diet 6 contained both the 0.2% inositol and 0.1% p-aminobenzoic acid, all such additions being at the expense of the dextrose. The p-aminobenzoic acid was increased to 0.2% during the reproduction and lactation periods.

In ration 7 the variable of interest was a commercial vitamin-free casein [6], this constituting 22.5% with a corresponding change in the carbohydrate component of the ration.

[2] Cerelose.
[3] Thoroughly extracted with 95% ethanol.
[4] Table 1.
[5] See footnote 2.
[6] Supplied by the S.M.A. Corporation.

Rations 8, 9, 10 and 11 represented tests of diet 1 with 0.2% yeast nucleic acid and the further addition of either 0.2% inositol or 0.1% p-aminobenzoic acid or both.

Diets 12, 13, and 14 differed from diet 1 in the following respects: instead of 25% fibrin being used as the protein, a combination of 10% fibrin, 10% washed casein [7] and 0.2% cystine was employed. All three diets contained 0.1% ascorbic acid. Diet 12 had 1% liver concentrate, diet 13 had 2% dried grass, and diet 14 contained 2% dried liver, all of these changes from diet 1 being at the expense of the carbohydrate component, which was dextrin.

EXPERIMENTAL

This study was conducted with five groups of animals. Groups 1, 2, and 3 received inositol and p-aminobenzoic acid separately from the ration. Inositol was administered in 10 to 15 mg. daily doses per animal until mating, and in 30 mg. daily doses during the reproduction and lactation periods. p-Aminobenzoic acid was fed in 3 mg. daily doses per animal during the first 4 weeks, followed by 7.5 mg. daily doses until breeding, and 15 mg. daily doses during pregnancy and lactation. Groups 4 and 5 received inositol and p-aminobenzoic acid in the rations, as shown in table 2.

Groups 1, 2, and 3. It will be noted from table 2 that inositol produced a very marked harmful effect on lactation when present in rations containing 2% dried grass, 1% liver concentrate, and 2% dried liver, respectively. On the other hand, p-aminobenzoic acid exerted a most pronounced protective action against inositol injury, as evidenced from efficiency in rearing of young. On ration 13 there was a change from 0 to 39%; on ration 12, from 15.9 to 61.0%; and on ration 14, from 9.4 to 85%.

Group 4. Because of the claim of Nakahara, Inukai, and Ugami ('41) that yeast nucleic acid may be a component of their L_2 factor, the rations in this group of experiments were fortified with 0.2% of such product.[8] In this experimental set-

[7] Thoroughly extracted with acidulated water and dilute ethanol.
[8] Obtained from Eastman-Kodak Co., Rochester, New York.

up p-aminobenzoic acid was added alone (diet 10) and in combination with inositol (diet 11). The injurious effect of inositol is again apparent. The supplementary effect of p-aminobenzoic acid is most marked. However, while there was protection against inositol injury in the presence of the latter, p-aminobenzoic acid, in group 4, did not show its suplementary role.

Group 5. The most surprising results were secured in this group of experiments. Previously, on the basal ration, in the absence of natural foods or the ''Bx'' factor, the lactation efficiency was about 6% when the protein was furnished by either 20% blood fibrin or by 10% casein and 10% blood fibrin, fortified with 0.2% cystine; also when dextrin was the source of carbohydrates, and when 4% of Sure's salts no. 1 (Sure, '41) provided all the minerals. Ration 2, containing 25% blood fibrin, dextrose as the source of carbohydrates, and 5% of Sure's salts no. 2, allowed 40% success in rearing of young. In other words, this radical change in the basal ration resulted in an increase of efficiency of 34% in lactation. The first experiments with this new basal ration were conducted with five females. This was later extended and results were obtained on fifteen mothers which bore thirty-two litters of 340 young. Another group was then started with fifteen females on the same type of ration but dextrin replaced glucose. While it is not possible at this time to make a fair comparison between the efficiency of glucose versus dextrin for lactation, since the mothers on the glucose diets had second litters while those on dextrin rations had only first litters, still the indications are already apparent that glucose is superior to dextrin for lactation, perhaps by allowing greater efficiency of bacterial synthesis of biotin, folic acid or unknown components of the vitamin B complex (Martin, '42b).

A comparison for lactation was also made between pure vitamin-free casein (diet 7) and blood fibrin (diet 2) using glucose and 5% of Sure's salts no. 2. From the results given in table 2 it is evident that casein, even when fortified with cystine, proved an inferior protein to blood fibrin for lactation.

The substitution of 30% glucose with an equivalent amount of highly milled rice (polished) increased the efficiency of rearing of young from 40.3 to 66.6%. Evidently the polished rice furnished an unknown factor or factors of the vitamin B complex essential for lactation. The addition of 0.2% inositol reduced lactation efficiency by about 50%. However, the addition of 0.2% p-aminobenzoic acid increased lactation efficiency to practically complete success. Also, addition of 0.2% p-aminobenzoic acid to a ration containing 0.2% inositol not only counteracted the harmful effects of the latter but showed almost all of its supplementary effect.

Growth experiments with p-aminobenzoic acid and inositol

The influence of p-aminobenzoic acid on growth of weaned albino rats, with initial weights of 34 to 46 gm., was studied by the paired feeding method on nine pairs of females and twelve pairs of males for 100 to 120 days. This study was carried out on a purified synthetic diet containing 18% vitamin-free casein. The daily dose of the aromatic acid was 3 mg. to the positive control animals. The daily doses per animal of the rest of the components of the vitamin B complex were as follows: 20 µg. thiamine, 20 µg. riboflavin, 20 µg. pyridoxine, 6 mg. choline chloride, 0.5 mg. nicotinic acid, 100 µg. calcium pantothenate, and 10 mg. inositol. The growth was excellent but there was no additional growth produced by the administration of p-aminobenzoic acid. Similarly, inositol addition, in the presence of p-aminobenzoic acid produced no growth responses in seven pairs of females and eleven pairs of males during a period of about 4 months, but there was no injurious effect on growth precipitated by inositol, as has been observed in lactation. The daily dose of inositol to the positive controls was 10 mg. These results are in harmony with those of Unna, Richards and Sampson ('41), Emerson ('41), and Martin ('42a). The reason for p-aminobenzoic acid being essential for lactation and not for growth of the weaned rat is not clear at present, unless there is a difference in the efficiency of synthesis of this substance between very small

nursing young and weaned rats whose capacity for synthesis has been established at that age and weight.

It is of interest to note that Martin ('42a), working with growing animals and the author with lactating animals have noticed dietary disturbances following inositol addition to the vitamin B complex mixture, which however, were counteracted by administration of p-aminobenzoic acid. Martin offers the hypothesis that inositol stimulates the growth of microorganisms which utilized and destroy some member of the vitamin B complex, known or unknown, thus precipitating a deficiency of that factor. It is also possible, however, that excess inositol may interfere with the synthesis of biotin, folic acid, or some other unknown member of the B complex. The response of p-aminobenzoic acid could then be interpreted as supplying a factor essential for synthesis of such substances as biotin and folic acid rather than for neutralization of a toxin (Martin, 42b). The syndrome produced by Martin following addition of p-aminobenzoic acid to the Rockland strain black rat was not confirmed by the author's observations on the weaned or lactating Wistar strain albino rat.

SUMMARY

p-Aminobenzoic acid has a markedly favorable influence on lactation of the albino rat. On the other hand, inositol has a pronounced injurious influence on lactation, which is counteracted by p-aminobenzoic acid.

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THE PANTOTHENIC ACID REQUIREMENT OF HENS FED A HEATED DIET

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Gillis, Heuser and Norris ('41, '42) reported that the fertile eggs of hens fed adequate amounts of all known vitamins except pantothenic acid failed to hatch unless the diet was supplemented with this vitamin. Taylor, Thacker and Pennington ('41) presented evidence showing that pantothenic acid has a beneficial effect on embryonic development during the first 14 days of the incubation period. These workers increased the pantothenic acid content of eggs either by direct injection of the vitamin into the egg or by supplementing the normal diet of the hens with additional amounts. Snell and Quarles ('41) studied the vitamin content of eggs during different stages of incubation and concluded that the chick embryo has the ability to synthesize nicotinic acid and inositol but not pantothenic acid and riboflavin.

The results of these studies demonstrate that pantothenic acid is required by the hen for reproduction, but no evidence has been presented showing the amount of the vitamin required for this purpose. Accordingly, studies designed to determine this have been conducted. The results of these experiments are reported in this paper.

EXPERIMENTAL PROCEDURE

The basal diet used in these studies was the same as that reported by Gillis et al. ('42) except that it contained the

equivalent of 1% alcohol soluble liver extract, heated to destroy pantothenic acid. The cereals in this diet were heated at 125°C. for 48 hours. This diet was found to contain 210 µg. of pantothenic acid per 100 gm. by the microbiological assay method of Pennington, Snell and Williams ('40). In this paper, however, the results of the assay will be considered to be 200 µg. per 100 gm. of diet.

White Leghorn pullets in their first year of production were used as the experimental subjects. Studies were made during two experimental periods, the first of 12 weeks' duration and the second of 10 weeks' duration. For the first experiment five lots of pullets were selected for uniformity and placed in wire-floored pens. The basal lot contained thirty pullets and the other lots fifteen pullets each. Males were rotated among the different lots frequently enough to maintain uniformity with respect to breeding. Eggs were incubated weekly and examined for fertility and embryonic mortality on the seventh, fourteenth and eighteenth days of incubation.

The basal diet was supplemented with d-calcium pantothenate in quantities sufficiently great to provide the amount of pantothenic acid per lot as outlined in table 1. The additions to the basal diet were made at intervals of about 5 days in order to keep destruction in storage at a minimum. The hens were changed directly from a normal diet to the experimental diets.

Following the first experimental period of 12 weeks, ten hens from the basal lot were continued on the basal diet. The rest of the hens were again placed on a well-balanced normal diet for 4 weeks. This allowed the hens to recover from any ill-effects due to possible deficiencies resulting from the consumption of the heated diet. At the end of this 4 weeks' period these hens were redistributed into five lots as equally as possible on the basis of the hatchability records of the previous experiment. The management of the hens and the experimental procedure used in this experiment were the same as in the previous one.

TABLE 1

Percentage hatchability of fertile eggs from hens fed different levels of pantothenic acid.

WEEK AND HATCH	MICROGRAMS OF PANTOTHENIC ACID ADDED PER 100 GM OF BASAL DIET						
	None	250	500	1000	1500	2000	2500
	%	%	%	%	%	%	%
				Experiment 1			
1	79(44)[1]	69(16)	80(5)	74(31)	——	70(29)	——
2	68(75)	80(34)	76(37)	62(34)	——	70(41)	——
3	56(57)	38(29)	47(46)	69(31)	——	72(39)	——
4	37(73)	33(33)	58(45)	60(15)	——	70(32)	——
5	33(72)	37(32)	64(56)	60(34)	——	83(40)	——
6	20(44)	21(38)	67(55)	58(33)	——	85(35)	——
7	10(63)	32(40)	53(55)	71(35)	——	71(42)	——
8	10(60)	37(30)	55(51)	63(38)	——	69(35)	——
9	7(46)	25(24)	66(47)	65(34)	——	65(30)	——
10	8(24)	20(25)	61(31)	76(33)	——	74(27)	——
11	9(12)	12(16)	48(25)	69(36)	——	79(19)	——
12	11(60)	26(31)	55(47)	57(37)	——	67(27)	——
Ave. (after depletion[2])	9.2	28.1	57.4	64.8	——	73.5	——
WEEK AND HATCH	NONE	250	500	1000	1500	2000	2500
				Experiment 2			
1	4(28)	——	60(30)	95(23)	67(27)	82(34)	77(31)
2	4(26)	——	55(29)	82(23)	65(26)	70(27)	89(27)
3	17(23)	——	67(31)	71(28)	80(30)	71(24)	80(35)
4	20(10)	——	54(24)	70(20)	81(31)	65(20)	77(28)
5	9(22)	——	67(27)	75(20)	79(24)	80(20)	77(30)
6	8(13)	——	56(25)	54(17)	90(21)	75(24)	69(29)
7	8(25)	——	33(27)	50(18)	74(23)	69(26)	71(32)
8	3(33)	——	16(37)	40(20)	48(27)	62(21)	49(35)
9	15(27)	——	22(37)	22(22)	58(26)	52(27)	47(19)
10	8(26)	——	27(37)	32(22)	45(22)	42(12)	43(21)
Ave. (3-10 inclusive)	11.0	——	42.8	51.8	69.4	64.5	64.2

[1] Data in parentheses represent number of fertile eggs incubated.

[2] Hatches 7-12, inclusive, for the lot not receiving pantothenic acid; for other lots hatches 3-12, inclusive.

RESULTS AND DISCUSSION

The results of feeding different levels of pantothenic acid on the hatchability of fertile eggs are summarized in table 1. In the first experiment the hatchability of the fertile eggs from the basal lot declined from 79% to about 10% in 6 weeks. After this there was no further significant decrease. This shows that the hens were completely depleted of their body reserves of pantothenic acid within this time. The average hatchability of the last 6 weeks, therefore, is taken as the average for this lot. All other lots received supplementary pantothenic acid and, hence, the amount stored in the eggs became stationary earlier. An inspection of the data indicates that the hens were significantly influenced by their previous dietary regime for only about 2 weeks. It was decided, therefore, to use the last 10 weeks of this experiment as the basis of the average figures for the other lots.

The decline in hatchability of the fertile eggs of the lot receiving the addition of 250 µg. of pantothenic acid was not quite as marked as in the basal lot, but it was so much below that of the other lots that there was no doubt that the 250 µg. of supplementary pantothenic acid was inadequate to maintain the hatching power of the eggs. The average hatchability of eggs from the three lots receiving the higher pantothenic acid additions was correlated with the level of pantothenic acid in the diet, the best hatchability being secured from the lot receiving the most pantothenic acid.

Although the differences in average hatchability of the three lots receiving the higher pantothenic acid additions appeared to be significant, it was felt advisable to examine the results statistically. This treatment showed a very significant difference between these lots. The data were also tested statistically for regression and were found to exhibit a significant regression. These results tentatively placed the optimum pantothenic acid requirement for reproduction in the hen above 1200 µg. per 100 gm. of the diet. This is in agreement with a previous experiment in which the authors ('42), with-

out attempting to determine quantitative requirement, secured better hatchability when the hens received a supplement of 2000 μg. of calcium pantothenate per 100 gm. of diet, than when the supplement was 1000 μg. per 100 gm.

The second experiment was designed to establish the quantitative requirements within a narrower range, and especially to establish an upper limit which the preceding experiment failed to do.

The results of experiment 2, as given in table 1, also showed that the pantothenic acid requirement for reproduction is above 1200 μg. per 100 gm. of the diet, thus being in accord with experiment 1. The average hatchability of the eggs from the lot receiving 1200 μg. of pantothenic acid per 100 gm. of diet was below the three lots receiving more pantothenic acid, although at the beginning of the period it had the highest hatchability of any lot. It is also clear from the results of this experiment that the optimum pantothenic acid requirement for hatchability does not lie above 1700 μg. per 100 gm. of diet. The hatchability of eggs from the lot receiving 1700 μg. was slightly better than that of the lots receiving more pantothenic acid.

The quantitative pantothenic acid requirement for maximum hatchability of fertile eggs in hens fed the diet used in these studies, therefore, is between 1200 and 1700 μg. per 100 gm. of diet. Average daily food consumption was 110 gm. per hen, which makes the average daily requirement per hen between 1320 and 1870 μg. The average weight of the hens was 1707 gm. On the basis of body weight the requirement would thus be between 775 and 1000 μg. per kilogram of hen per day. The fact should not be overlooked, however, that the requirement may be less if hens are fed a more nearly normal diet of unheated feedstuffs, since a sufficiency of other necessary factors may exercise a sparing effect on pantothenic acid.

The basal diet used in these studies appeared to be deficient in some factor (or factors) required for reproduction other than pantothenic acid. This deficiency became serious, however, only after the hens had been maintained on this diet

for a prolonged period of time. The rapid drop in hatchability of all lots during the last few weeks of the second experiment is evidence of the existence of this latent deficiency. The decline was too great to be accounted for on the basis of seasonal variations alone. Cravens, Sebesta, Halpin, and Hart ('42) have recently reported biotin to be essential for hatchability. Since biotin has been shown by Ringrose and Norris ('36) and by others to be slowly destroyed by dry heat treatment, it is possible that the diet may also have been partially deficient in this factor.

The effect of pantothenic acid on the viability of the chicks hatched in both experiments was marked. The chicks hatched from lots receiving less than 1200 µg. of pantothenic acid were inferior. They usually exhibited general debility, muscular incoordination, swollen hocks, and poor down quality. Many of the embryos had great difficulty in breaking through the shell and died soon afterwards. When chicks from deficient lots were placed on an adequate diet mortality was extremely high. On the other hand, the chicks hatched from the lots receiving more adequate levels of pantothenic acid had little mortality and grew vigorously when given an adequate diet.

The peak of embryonic mortality in the lot fed the basal diet occurred near the end of the incubation period. In this lot 79% of all embryonic deaths occurred after the fourteenth day, and 52% occurred after the eighteenth day. Up to the fourteenth day the differences in the total percentages of dead embryos in the different lots were slight. The mortality of the embryos from lots receiving adequate pantothenic acid was more uniformly distributed over the incubation period.

The effects of the different levels of pantothenic acid on egg production were also studied. During the first experiment the average rate of egg production was best when the hens received a supplement of 500 µg. or above. During the second experiment the pantothenic acid content of the diet could, in no way, be correlated with rate of egg production. It is impossible to say from these results, therefore, whether or not pantothenic acid influences egg production, but it appears

that, in any case, 700 µg. per 100 gm. of diet is sufficient for this function.

The mortality and body weight of the hens were not influenced by the level of pantothenic acid in the diet. Apparently the requirement for maintenance in the mature hen is not greater than the pantothenic acid content of the basal diet which was approximately 200 µg. per 100 gm. This observation indicates that the pantothenic acid requirement of chickens decreases with advancing age, as Bauernfeind, Norris and Heuser ('42) found that the quantity of pantothenic acid required for chick growth is about 600 µg. per 100 gm. of diet. Waisman, Mills and Elvehjem ('42) reported that the pantothenic acid requirement for maximum chick growth is 750 µg. per 100 gm. of diet. These results are in agreement with the conclusion of Unna and Richards ('42), that the amount of pantothenic acid required by the adult rat is not more than 25% of that needed for early growth.

CONCLUSIONS

Hens fed a diet consisting principally of heated feedstuffs supplemented with known necessary minerals and vitamins except pantothenic acid were found to require between 1200 and 1700 µg. of this vitamin per 100 gm. of diet in order to maintain optimum reproduction. The pantothenic acid requirement for egg production was found to be not more than 700 µg. per 100 gm. of diet and may be considerably less. Maintenance of weight and livability were satisfactory on the basal diet which contained 200 µg. of pantothenic acid per 100 gm.

The basal diet used in these studies was found to be deficient in a factor (or factors) required for reproduction other than pantothenic acid. The deficiency affected reproduction detrimentally, however, only after the hens had been maintained on the diet for a prolonged period of time. Whether or not the deficiency influenced the requirement for pantothenic acid could not be determined from the evidence.

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THE INADEQUACY OF EIGHT SYNTHETIC B VITAMINS FOR THE NUTRITION OF PUPPIES — UNKNOWN FACTOR (FACTORS) IN YEAST AND PROBABLY LIVER [1] [2]

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ONE FIGURE

This investigation was made in an attempt to determine whether dogs would grow normally on a diet containing only the chemically pure vitamins available at the time the work was done (1941–42). In some previous unpublished work it was noted that adult dogs developed ulcers in the mouth and on the leg joints while subsisting on similar diets. Since these dogs bore jejunostomies, there was the possibility that the lesions were the result of discharges from the stoma of acid chyme which was incompletely removed by licking. Leg joint lesions were demonstrated by Cowgill, Stucky and Rose ('29) in dogs deprived of the entire vitamin B-complex. In order to get further information concerning the inadequacies of this diet, it was fed to puppies from the time of weaning.

METHODS

Seven mongrel puppies from three litters were used in this investigation; dogs 1 and 2 were from one litter, dogs 3 to 6, inclusive, from a second, and dog 7 from a third litter. The

puppies received the diets shown in table 1. The salt mixture was that of Arnold and Elvehjem ('38) except that cobalt was added in such quantities that each dog received 0.1 mg. per day (Frost, Elvehjem and Hart, '41).

The vitamin contents in milligram per gram of the dried foil yeast [3] were as follows: thiamine 0.108; riboflavin 0.040; nicotinic acid 0.259; pyridoxine 0.020; and pantothenic acid 0.180.

The casein was freed of vitamin B-complex by repeated extractions with 0.1% acetic acid and ethyl alcohol. The extraction was assumed to be complete when the fluorescence due to riboflavin disappeared from the filtrates viewed in ultra-violet light in the absence of visible light. The sucrose was extracted twice with 80% (by weight) ethyl alcohol and washed after each extraction with a like mixture while on the filter.

A diet containing the casein and sucrose, extracted according to these methods, was compared in growth studies on rats with an identical diet made with a commercial vitamin B-complex-free casein.[4] The paired feeding technic revealed the diets to be indistinguishable and free of vitamin B-complex.

The protein of yeast in the basal diet was replaced by casein in the experimental diet. During the experimental period the animals received a water solution or suspension of the synthetic crystalline B-vitamins in amounts roughly equivalent to the 10% high vitamin yeast supplement in the basal diet. Some of the vitamins were fed in somewhat larger or smaller amounts than furnished by the yeast to produce better agreement between the supplement and the published requirements for the dog.

All the puppies used in this investigation were weaned at the age of 6 weeks and placed on their respective diets. During the early part of the experiment the dogs were immunized against distemper, dewormed and freed of fleas.

[3] Fleischmann's.
[4] S. M. A. Corporation, Chagrin Falls, Ohio.

RESULTS

Dog 1 was maintained on the basal diet for 151 days and at no time showed any signs of deficiency. He was exceptionally active, always ate willingly, and grew at a regular and uniform rate. On the 151st day he was placed on the experimental diet and maintained thereon for 105 days (fig. 1). Near the 75th
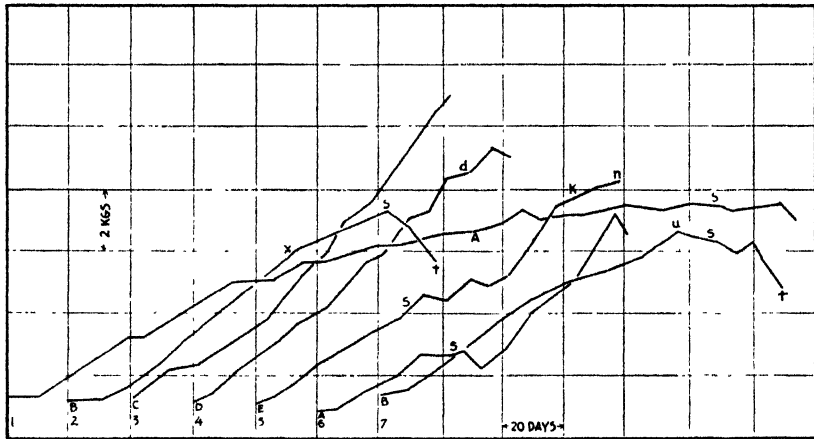
FIGURE I
WEIGHT CURVES OF PUPPIES 1-7 INCLUSIVE (KILOGRAMS)

Fig. 1 Growth curves of dogs, fed synthetic diets containing all fat-soluble vitamins and synthetic B factors, with and without yeast or liver filtrate concentrates.

Dog 1 A, placed on experimental diet A, containing all eight synthetic B factors; s, scaliness of skin appeared.

Dog 2 B, placed on experimental diet B, i.e., containing all synthetic B factors shown in table 1, except inositol and p-aminobenzoic acid, plus yeast fraction II of Osborne and Wakeman ('19); x, yeast fraction discontinued; s, scaliness of skin and diarrhea appeared; + death.

Dog 3 C, dog placed on experimental diet C, i.e., all eight synthetic factors, plus yeast filtrate factor of Koehn and Elvehjem ('37) — no deficiencies.

Dog 4 D, dog placed on experimental diet D, i.e., all eight synthetic factors and a filtrate factor from liver (Koehn and Elvehjem '37); d, skin dry and dusty but not scaly.

Dog 5 E, placed on experimental diet E, i.e., double amounts of all eight synthetic B factors shown in table 1; s, scaliness of skin appeared and intermittent diarrhea; k, placed on kitchen scraps; n, skin normal.

Dog 6 A, dog on diet A throughout; s, scaliness of skin and diarrhea continuous and severe, + death.

Dog 7 B, placed on diet B without filtrate factor; u, ulcerous dermatitis particularly on ventral surfaces. At this point inositol and p-aminobenzoic acid added; dermatitis persisted; s, scaliness of skin became general after 102nd day; diarrhea throughout entire time; + death.

day on the experimental diet the dog became coprophagous, showed loss of hair and a small amount of scaliness of the skin. At the end of the basal period the hematocrit was 34% and the hemoglobin concentration 14.9 gm. per 100 ml. of blood. During the last 75 days the dog received 200 mg. of iron in the form of ferric ammonium citrate in addition to that furnished by the salt mixture in the diet. On the 100th day after starting the experimental diet the hematocrit was 42% and the hemoglobin concentration 17.0 gm. per 100 ml. of blood.

Dog 2 received the experimental diet minus inositol and p-aminobenzoic acid. An additional supplement was given in the form of a yeast fraction prepared by the method outlined for fraction no. II, by Osborne and Wakeman ('19). This fraction was fed in amounts equivalent to twice the yeast received by dog 1. On the 73rd day the Osborne-Wakeman fraction was discontinued and thereafter the dog showed a decline in rate of growth (x, fig. 1). On the 102nd day the dog's eyes began to discharge a great deal of mucoid material, but abnormal secretions from mouth and nose were absent. At this time the dog also began to suffer from intermittent diarrhea. The skin had become so dry and scaly that the coat of hair was very dusty (s, fig. 1). From the 102nd day until the 118th day the dog continued to eat about half of its usual dietary intake and lost weight rapidly. During this period of rapid weight loss inositol and p-aminobenzioc acid were added to the vitamin supplement. On the 117th day the hematocrit was 27% and the hemoglobin concentration 10.8 gm. per 100 ml. of blood.

Dog 3 received the experimental diet plus an additional supplement of a yeast concentrate prepared according to the method for the preparation of the filtrate factor as outlined by Koehn and Elvehjem ('37). The starting material was 400 gm. of 50% alcohol (by weight) — soluble extract of dry yeast. The daily dose was equivalent to 20 gm. of the original yeast. Dog 3 showed the best rate of growth of all the dogs. At no time did any of the previously mentioned signs of deficiency appear. The skin was clean and the hair clean and glistening. The yeast concentrate, therefore, contains the additional fac-

tor or factors required for normal growth and maintenance of healthy skin. This dog was also fed daily 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. On the 89th day the hematocrit was 31% and the hemoglobin concentration 12.0 gm. per 100 ml. of blood.

Dog 4 received the experimental diet plus an additional supplement of a liver concentrate which was made from 400 gm. of 95% alcohol-soluble liver extract as outlined by Koehn and Elvehjem for the preparation of the filtrate factor. The daily dose was equivalent to 4 gm. of the original liver extract. This dog also received daily 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. This dog showed a good rate of growth until about the 82nd day, at which time the rate began to decrease a little. About the 90th day it was observed that although the skin could not be considered scaly, it was dry and dusty (d, fig. 1). The coat of hair was still in excellent condition. Apparently a liver fraction prepared in this manner does not contain sufficient amounts of the missing factor or factors. On the 89th day this dog had a hematocrit of 33% and a hemoglobin concentration of 12.2 gm. per 100 ml. of blood.

Dog 5 received the experimental diet with double the dosage of vitamins shown in table 1. This dog also received an additional daily supplement of 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. About the 50th day of the experiment dry, scaly skin and dusty hair were observed. There were frequent interludes of remittant diarrhea. The areas which had been clipped for the distemper immunization injections showed a very slow rate of hair growth and there was definite achromotrichia. This was also found to be true of dogs 6 and 7, but not the other dogs. After 103 days on the diet this dog was changed to a diet of kitchen scraps (n, fig. 1) and in the short period of 2 weeks the skin was clean and the hair glistening; the clipped areas showed considerable new pigmented hair growth. On the 89th day the hematocrit was 31% and the hemoglobin concentration 12.0 gm. per 100 ml. of blood.

Dog 6 received the experimental diet and at no time appeared to be healthy. This dog also received daily 200 mg. of iron as ferric ammonium citrate. The dermatitis of dog 6 was not as severe as that of dog 7, but there was dryness and scaliness of skin, and the hair was dull, exceptionally dusty and sparse. This dog suffered from almost constant diarrhea. His mature appearance and behavior were marked characteristics.

TABLE 1

Composition of diets and vitamin supplements.

	BASAL		EXPERIMENTAL	
Casein	25%		35%	
Sucrose	41%		41%	
Hydrogenated cottonseed oil	18%		18%	
Bone ash	4%		4%	
Salts	2%		2%	
Yeast (dry)[1]	10%		0%	
Thiamine chloride hydrochloride [2]	0		0.050 mg./kg./day	
Riboflavin	0		0.100 mg./kg./day	
Nicotinic acid	0		0.500 mg./kg./day	
Ca-d-pantothenate	0		0.220 mg./kg./day	
Pyridoxine	0		0.040 mg./kg./day	
Choline chloride	0		0.200 mg./kg./day	
Inositol	0		0.500 mg./kg./day	
p-Aminobenzoic acid	0		0.500 mg./kg./day	
Vitamin A (concentration; 200,000 I.U./gm.)	200	I.U./kg./day	200	I.U./kg./day
Vitamin D (viosterol in oil)	10	I.U./kg./day	10	I.U./kg./day
Vitamin E	1.0	mg./kg./day	1.0	mg./kg./day
Vitamin K	1.0	mg./kg./day	1.0	mg./kg./day

[1] Kindly furnished by Standard Brands, Inc.

[2] All vitamins except A, D, inositol and p-aminobenzoic acid were kindly furnished by Merck and Company, Rahway, New Jersey.

On the 96th day this dog showed little interest in his food but retained his normal, although mild, degree of activity. On the 98th day he died and autopsy revealed the thymus gland heavily spotted with hemorrhagic areas about 2 to 5 mm. in diameter. A similar observation was made by McKibbin et al. ('39), Schaefer et al. ('42) and Morgan and Simms ('40a,

'40b), and ascribed to pantothenic acid deficiency because prophylaxis was achieved by the administration of 0.1 mg. of this vitamin per kilogram per day. Dog 6 received 0.22 mg. of Ca-d-pantothenate per kilogram per day which is apparently more than the growing dog requires. On the 89th day the hematocrit was 31% and the hemoglobin concentration 11.7 gm. per 100 ml. of blood.

Dog 7 received the experimental diet, except that inositol and p-aminobenzoic acid were omitted. About the 10th day, dog 7 showed small ulcers on the ventral surface of its body; these disappeared on the 82nd day. On the 96th day new areas appeared and dermatitis was general (u, fig. 1). At this time the dog began to lose weight and the vitamin supplement was changed by the inclusion of inositol and p-aminobenzoic acid. On the 102nd day the dermatitis was extensive and the dog's eyes began to discharge considerable amounts of mucoid material. On the 120th day the whole ventral surface and the inside areas of the dog's legs were spotted with small ulcers. The rest of the animal's skin was in a dry, scaly and dusty condition. The dog's coat of hair, which until the 102nd day was glistening, had become dull and dirty by the 120th day. During the whole experiment the dog suffered from diarrhea. Dog 7 died on the 127th day; autopsy failed to reveal any specific cause for death. On the 120th day the hematocrit was 23% and hemoglobin concentration 7.1 gm. per 100 ml. of blood.

DISCUSSION

The above investigation strongly suggests that the lesions observed in the adult jejunostomized dogs were due to leakage from the stoma. Neither the leg joint lesions nor the mouth lesions were found in any of the puppies, but there is the possibility that this difference in response might have been due to the difference in the ages of the experimental animals. That the lesions might have been due to a deficiency of inositol or p-aminobenzoic acid seems to be ruled out on the basis of their failure to appear in dogs 2 and 7. The small lesions seen in

dog 7 were not the same as seen in the adult dogs and were not confined to the joints.

It appears that there is in yeast one or more substances concerned with the maintenance of healthy skin and hair in dogs. Morgan ('41) reported that a young dog fed a synthetic diet supplemented with a vitamin B-complex containing thiamine chloride hydrochloride, riboflavin, pyridoxine, pantothenic acid, and nicotinic acid, grew well and showed no greying hair after 6 months on the diet. However, she reports that this dog had dull powdery hair. Morgan used wheat germ oil instead of synthetic α-tocopherol as a source of vitamin E, and choline chloride, inositol, and p-aminobenzoic acid as such were included.

Schaefer, McKibbin, and Elvehjem ('42) demonstrated that when a diet containing acid and alcohol-extracted casein, supplemented with thiamine chloride hydrochloride, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and choline chloride, was fed to young dogs a deficiency characterized by anorexia and loss of weight appeared after 1 to 3 months. They also observed greying of hair in some dogs. They state that addition of inositol, p-aminobenzoic acid or glutamine had little or no effect on these symptoms. These workers do not report the occurrence of dermatitis in their animals.

Since the animals used in this investigation received far less choline than administered by Schaefer, McKibbin and Elvehjem ('41) it may be argued that they lacked this factor. Some of our dogs showed weight losses at about the same time after the experimental diets were begun as did those reported by these other observers. The additional fact must not be overlooked that the animals used in this investigation received 35% of their diet as casein. On the basis of the onset of weight loss these two experiments seem comparable. Furthermore, gross examination of the dogs that came to autopsy showed no abnormalities in the livers.

Those dogs showing the most severe dermatitis, dusty hair and diarrhea were the ones which lost weight and died. Since pronounced diarrhea was observed in only those dogs receiv-

ing the quantities of vitamins listed in table 1, and was not an outstanding characteristic of the dog receiving twice these amounts, the suggestion is made that although the quantities administered probably exceeded the minimal requirements, they may not be optimal for all of the components. The vitamin supplement was lower than the amounts in the yeast of the basal diet by three-fourths in thiamine chloride hydrochloride, and by one-half in pantothenic acid. The feeding of 0.5 mg. per kilogram per day of inositol and p-aminobenzoic acid was purely arbitrary since little was known concerning the dog's requirements for these compounds.

The above observations seem to justify the conclusion that the factor or factors responsible for the maintenance of good health of the skin and hair in growing dogs is found in yeast and probably liver, and that the development of signs of deficiency disease is not due to a deficiency of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, inositol or p-aminobenzoic acid. Furthermore, it appears to be essential to make thorough extractions of both protein and carbohydrate components of the diet in experiments of this type.

SUMMARY

Puppies were fed synthetic diets supplemented with a synthetic vitamin B-complex with or without additional vitamin-rich concentrates.

The young dogs receiving the synthetic vitamin B-complex containing only thiamine chloride hydrochloride, riboflavin, nicotinic acid, Ca-d-pantothenate, pyridoxine, choline, inositol and p-aminobenzoic acid developed varying degrees of dermatitis, loss of hair and general unhealthy skin and coat in 75 to 125 days, and died in 100 to 150 days.

If dry whole yeast or concentrates of yeast or liver were fed in addition to the crystalline vitamins the deficiency failed to appear.

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ANTI-ACRODYNIC POTENCY OF SEED OILS [1] [2]

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The curative effect of certain oils on rat acrodynia has
been demonstrated by Birch and György ('36), Richardson
and Hogan ('36), Quackenbush, Platz and Steenbock ('39),
and others. Linoleic acid was an active ingredient in these
oils while linolenic acid was apparently inactive since linseed
oil, containing a total of 70% linoleic and linolenic acids, was
much less curative than corn, cottonseed, or wheat germ oil
with a total of 40 to 55% linoleic acid and no linolenic acid.
However, it was possible that fatty acids other than linoleic
might be curative and that certain oils might be more po-
tent than any yet studied.

The work presented in this paper was an attempt to cor-
relate the observed anti-acrodynic potency of various seed
oils with the linoleic acid content as determined on identical
samples. It does not deal with the cure of the less severe
dermatitis of Burr and Burr ('29).

EXPERIMENTAL

I. Methods and techniques

The technique employed for determining the potency of the
oils was that previously developed in this laboratory (Quack-
enbush, Platz and Steenbock, '39). Female rats and their

litters were placed on a diet consisting largely of potatoes until the young attained a weight of 40 gm. The young were then transferred to a diet having the following percentage composition: glucose [3] 78, water and alcohol-extracted casein [4] 18, and salts 4. This was supplemented daily with the following vitamins: thiamine 10 µg., riboflavin 20 µg., carotene 10 µg., and calciferol 1 µg. per rat per day. With this diet (diet V) acrodynia was produced in our specially prepared rats in 5 to 6 weeks.

The severity of the dermatitis at each of the loci of incidence (mouth, eyes, paws, ears and tail) was evaluated on a scale of four. The total score on each animal was then used to give the dermal index (Quackenbush, Steenbock, Kummerow and Platz, '42).

When an animal had a dermal index of 5 to 7 it was given a daily supplement of the oil to be tested. After 3 weeks an animal was considered cured if the lips, eyes and ears were normal in appearance and the paws and tail were free from all symptoms except a dry scaliness. This was equivalent to a dermal index of 2 or less. When a dose produced a dermal index of 2 or less in more than 50% of the rats in the group it was accepted as being curative.

All the oils were fed daily in amounts equivalent to 12 mg. of linoleic acid. This was considered ample since the minimum curative level of linoleic acid in the form of the ethyl ester had been found to be between 5 mg. and 10 mg. per rat per day (Quackenbush, Platz and Steenbock, '39). Some of the oils which were curative at the 12 mg. level were later fed in amounts equivalent to 4 mg. of linoleic acid.

The linoleic acid content of most of the oils was determined by a method developed in this laboratory (Anthony, Quackenbush and Steenbock, '43). For the remaining analyses we are indebted to Schuette and co-workers ('30, '32, '34a, '34b, '37, '40a, '40b, '40c, '41). Analysis of identical samples was considered necessary since the assays of some of the oils

[3] Cerelose.
[4] Labor for purification of the casein was furnished by the Works Progress Administration.

TABLE 1

The effect on rat acrodynia of seed oils of known composition. [1]

OIL	LINO-LEIC ACID	LINO-LENIC ACID	AMOUNT OF OIL FED					
			Equivalent to 12 mg. linoleic acid			Equivalent to 4 mg. linoleic acid		
			No. rats	No. cured	Average dermal index	No. rats	No. cured	Average dermal index
	%	%						
Tobacco [2]	81	0	3	3	1			
Hackberry [2]	70	0	4	4	1			
Muskmelon	70	0	4	3	2	4	1	3
Watermelon	66	0	7	6	2	3	1	2
Poppy	65	0	6	4	2	7	2	4
Raisin	63	0	5	5	1	4	3	2
Cucumber	61	0	4	3	2	3	0	4
Elderberry [2]	58	24	6	3	3			
Sunflower	53	1	5	3	2			
Pumpkin	49	0	7	5	2	1 [3]	1	2
Squash	49	0	8	7	2	2	0	4
Rye germ [2]	42	5	3	3	2			
Sesame	39	3	3	3	1	2	0	8
Fenugreek [2]	37	19	5	2	3			
Clover	36	18	6	4	2	3	1	4
Highbush cranberry [a]	36	0	4	3	2			
Hemp [4]	31	33	5	2	2	3	0	3
Oat germ [5]	31		4	4	1			
Linseed [4]	27	39	4	2	3			
Coffee bean [2]	25	0	4	4	1			
Alfalfa	22	42	6	3	3	11	4	4
Brazilnut [2]	21	0	4	4	1			
Perilla [4]	18	65	4	0	4			
Elm [2]	8	0	4	3	2			

[1] In the computation of this table negative results obtained on animals which contracted "respiratory" infections or which weighed less than 45 gm. when given the supplements were not included because such animals frequently failed to respond to supplements of known potency.

[2] We are indebted to Dr. H. A. Schuette and co workers for these samples and their analyses.

[3] Four of the rats put on experiment died early. They all had a severe acrodynia (average dermal index 5).

[4] These were also assayed at 36 mg. equivalency. Hempseed oil was curative (average dermal index of 2) while linseed and perilla oil were non-curative (average dermal index of 4 and 3, respectively).

[5] Oat germ oil not analyzed. Analyses listed are from the literature (Amberger and Hill, '27).

reported in the literature were found to vary over a wide range (Hilditch, '40; Dean, '38).

II. Results

Oils fed in an amount equivalent to 12 mg. of linoleic acid consistently cured acrodynia unless the oil contained a considerable amount of linolenic acid (table 1). The presence of linolenic acid interfered with the curative action as shown by the data on linseed, alfalfa, perilla, elderberry and fenugreek seed oils. When these oils were fed in amounts greater than the 12 mg. equivalency, the average dermal index was not reduced correspondingly. Hempseed and perilla oils fed at a level equivalent to 36 mg. of linoleic acid were only slightly more curative than at the 12 mg. level, while linseed oil actually was less curative at 36 mg. than at 12 mg. A few tests on marine oils indicated that lipids other than linolenic acid also interfered with the cure of acrodynia. In fact, shark liver, herring, seal, and whale oils aggravated the symptoms to such an extent that the average dermal indices of the groups at 3 weeks ranged from 8 to 15.

With the exception of the instances cited above, the oils had a potency approximately equivalent to their linoleic acid content. Doses equivalent to 12 mg. were curative while doses equivalent to 4 mg. were not curative in eleven out of twelve oils tested. The one exception was raisin seed oil, but the data with this oil are too limited to warrant final conclusions.

SUMMARY

1. Twenty-four seed oils of known linoleic and linolenic acid content were assayed for their potency in the cure of rat acrodynia.

2. The anti-acrodynic potency of the oils was found to be in direct proportion to their linoleic acid content unless they contained considerable amounts of linolenic acid.

3. When linolenic acid was present the curative effect of the oils was greatly reduced. Isolated data on marine oils

suggest that other lipids besides linolenic acid also produce this effect.

4. When linolenic acid was absent, or when it was present only in minor proportions, a daily dose equivalent to 12 mg. of linoleic acid cured the acrodynia; 4 mg. was not curative.

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THE UTILIZATION OF *d*-GLUCONO-δ-LACTONE BY THE ORGANISM OF THE YOUNG WHITE RAT

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d-Glucose is readily oxidized to *d*-gluconic acid by bromine in acid or neutral solution. Gluconic acid has also been isolated as a product of the oxidation of glucose by the enzyme glucose dehydrogenase of the liver (Harrison, '32). The occurrence of such a reaction in the intact living organism has, however, not yet been proven. Gluconic acid in aqueous solution forms an equilibrium mixture with its anhydrides, the γ- and δ-lactones. An oxidation product of gluconic acid is glucosaccharic acid.

CHO	COOH	CO—————	COOH
HCOH	HCOH	HCOH	HCOH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HC———— -O	HCOH
CH₂OH	CH₂OH	CH₂OH	COOH
d glucose	*d*-gluconic acid	*d*-glucono-δ-lactone	*d*-glucosaccharic acid
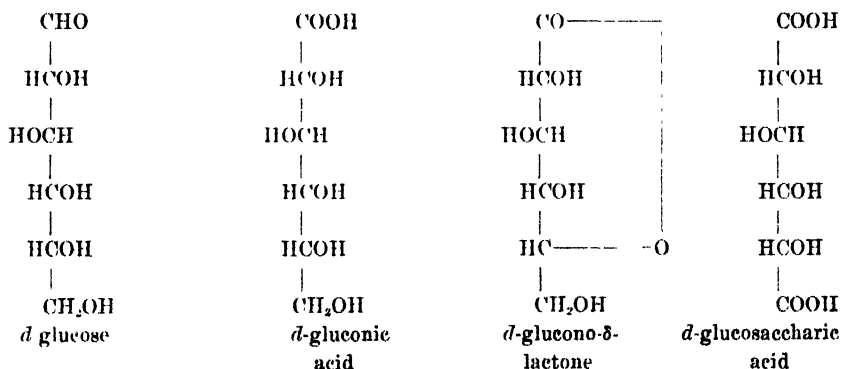
Little information is available as to the utilization of gluconic acid (or its lactones) in the animal organism. The evidence suggests the following metabolic changes: (1) Part of the administered gluconic acid may be oxidized to *d*-gluco-

saccharic acid, which has been isolated from the urine. The remainder is believed to undergo complete oxidation (Mayer, '02). (2) Part of the gluconic acid may be excreted in the urine unchanged (Baer and Blum, '11; Hermann, '30; Paderi, '15; and Schott, '11). (3) Part of the acid may be converted to glucose as evidenced by the formation of glucose when gluconic acid is incubated with liver tissue, and by an increased content of liver glycogen when the acid is fed to rabbits (Paderi, '15).

Gluconic acid may be converted to pentose in the laboratory (Ruff, 1899). A similar reaction in vivo has been suggested as an explanation of the origin of the pentose excreted in pentosuria, i. e., an abnormal oxidation reaction of glucose in this error of metabolism. It is also possible that gluconic acid may be an oxidation product of glucose which, in the normal organism, is further oxidized to carbon dioxide and water, but which, in the pentosuric, escapes further oxidation and is excreted as pentose. However, neither Salkowski (1899) nor Mayer ('02) could detect pentose in the urine of rabbits to which gluconic acid was fed.

Baumgarten ('05-'06) reported that gluconic acid was readily oxidized by both the normal and the depancreatized dog and also by the human diabetic, an observation which was not confirmed with rabbits by Hermann ('30). That gluconic acid was excreted largely unchanged has been assumed in studies of the value of gluconic acid as an acidifying agent for human urine (Gold and Civin, '39). When considerable amounts of the δ-lactone were fed to man under controlled experimental conditions, the urine became more acid. The acidifying value of the lactone under the usual conditions of diet and fluid intake was, however, believed to be slight.

The experimental studies have been limited by the lack of accurate quantitative methods for the determination of gluconic acid and its possible degradation products in tissues and biological fluids. Attempts have usually been made to isolate gluconic acid and glucosaccharic acid and their de-

rivatives by procedures which are far from giving quantitative results.

Our interest in gluconic acid was stimulated by recent industrial developments which have made the acid available commercially at a reasonable cost. Calcium gluconate has been used in therapeutics as an organic salt of calcium. Gluconic acid,[1] its lactone and salts, have many possible applications in the food industries. The lactone in aqueous solution forms an equilibrium mixture with the acid. At room temperature, after approximately 90 minutes, the solution contains about 20% of the lactone and 80% of the acid.

A direct method of study of the utilization of gluconic acid or its lactones, namely, the study of its utilization as a source of energy in the intact organism, has not been employed, so far as is known to us. If gluconic acid is oxidized to any significant extent, it should be able to furnish calories and thus to promote the growth of young white rats when added as a supplement to a diet whose essential deficiency is with respect to inadequate calorific value (Bendaña and Lewis, '35). The experiments detailed in the present paper represent such a study of the biological value of glucono-δ-lactone in the young white rat. Our results show that the addition of the lactone to the low-calorie diet was followed by an increased rate of growth which was similar to that observed when isocaloric amounts of a readily utilizable carbohydrate, glucose, served as the source of supplementary calories. The amount of the lactone which could be administered and utilized, however, was limited by its irritant action in the gastrointestinal tract, which resulted in diarrhea, as already observed by others (Mitchell, Cook and O'Brien, '39; Paderi, '15).

EXPERIMENTAL

Young white rats in litter units were used for the experiments. After weaning, they received a stock diet of milk,

whole wheat bread, and lettuce, or of a commercial rat chow. When the rats reached the weight of 65 to 80 gm., they were fed a basal diet low in calorific value (approximately 14.0 Cal. daily) in the restricted amounts shown in table 1. In most cases this diet permitted slight growth of rats of the weight used. Extra calories in the form of glucose monohydrate or glucono-δ-lactone were added as a supplement to the basal diet, and the effects on growth were observed. The various

TABLE 1

Basal and experimental diets.

	1	2	3
	gm	*gm*	*gm.*
Casein	1.2	1.2	1.2
Cod liver oil	0.1	0.1	0.1
Lard	0.1	0.1	0.1
Agar	0.1	0.1	0.1
Salt mixture [1]	0.4	0.4	0.4
Glucose	1.5	2.25	1.5
Glucono-δ-lactone			0.75
Daily food intake [2]	3.4	4.15	4.15
Daily calorie intake (calculated) [3]	14.0	17.0	16.6

[1] Osborne-Mendel salt mixture to which copper sulfate has been added to give a concentration of approximately 0.04% of copper.

[2] Each rat also received one dried yeast tablet daily (appproximately 400 mg.).

[3] Calculated on the basis of 3.5 cal. per gram of glucono-δ-lactone and of 1.4 cal. per yeast tablet.

diets are given in table 1. Although glucose was fed as the commercially available monohydrate, the equivalent amount of anhydrous glucose is presented in the diets as given in table 1. One gram of anhydrous glucose is equivalent to 1.1 gm. of the monohydrate. The calorific value of the lactone was stated by the manufacturers to be 3.5 Cal. per gram (Committee on Foods, '33). The extra calories of the supplementary glucose and gluconolactone were 3.0 and 2.6, respectively, amounts somewhat over 15% of the calorific value of the basal diet. In earlier experiments, an attempt was

made to add, as a supplement, 1.5 gm. of the gluconolactone daily. This resulted in a rather severe diarrhea, so that the amount of lactone was reduced to 0.75 gm. (diet 3). With this diet, no diarrhea of significance was observed.

All rats were maintained on the low-calorie basal diet for 6 to 7 days. At the end of this period, the rats of each litter were divided into three groups. The first group continued to receive the basal diet (diet 1); the second received the basal diet plus added glucose (diet 2); the third received the basal diet plus gluconolactone (diet 3). The animals were weighed every 3 days. Since the animals had similar nutritional histories at the beginning of the experiment, the average gains for the early periods seemed more significant, particularly since, as the animals gained in weight, the calorific value of the diet would be expected to be less adequate. The results of a period of 2 weeks (litter A) and 3 weeks (litter B) are summarized in table 2.

The results presented in table 2 show that the gluconolactone was almost as effective in the promotion of growth as was glucose. Thus with litter A, the average daily gains with supplementary glucose and gluconolactone were 208 and 213%, respectively, of that of the control group (basal diet); with litter B, the corresponding values were 217 and 191%.

Further evidence of the utilization of the gluconolactone is afforded by table 3. After the initial 2 or 3 week period, the diets were changed. The animals which had received the control diet (diet 1) were fed the gluconolactone supplement (diet 3), and those which had received extra calories as glucose (diet 2) or the lactone (diet 3) were placed on the control diet. The responses to these changes in diet were striking. The animals which had previously shown fair or good growth on diets 2 or 3 ceased to gain significantly and, for the most part, lost weight slightly. This loss in weight was not unexpected, since the basal diet supplied the minimal calories for the animals at the beginning of the experiments and could hardly be expected still to be adequate for the animals after fair growth had proceeded for 2 or 3 weeks. On the

other hand, the animals previously fed the basal diet grew satisfactorily when placed on the diet containing supplementary gluconolactone. After 2 weeks (period II) on the basal

TABLE 2

Growth of young white rats fed low-caloric diets with and without supplements.

Weights of rats fed low calorie diets and similar diets in which extra calories as glucose or gluconolactone were provided.

The feeding periods were 14 and 21 days for litters A and B, respectively.

RAT NO.	SEX	DIET	WEIGHT		CHANGE IN WEIGHT	TOTAL GAIN FOR GROUP	AVERAGE DAILY GAIN PER RAT
			Initial	Final			
			gm.	*gm.*	*gm.*	*gm.*	*gm.*
				Litter A			
11	F	1	82.9	87.6	+ 4.7		
16	F	1	64.6	72.2	+ 7.6	27.5	(0.65)
18	F	1	64.1	79.3	+15.2		
12	F	2	71.6	87.9	+16.3		
13	F	2	76.9	92.0	+15.1	56.7	(1.35)
19	M	2	69.1	94.4	+25.3		
14	F	3	73.5	90.0	+17.4		
15	M	3	70.0	95.2	+25.2	58.3	(1.39)
17	F	3	67.8	83.5	+15.7		
				Litter B			
22	F	1	70.6	82.4	+11.8		
23	M	1	69.5	76.6	+ 7.1	52.7	(0.62)
25	F	1	66.2	82.1	+15.9		
30	F	1	62.0	79.9	+17.9		
26	M	2	62.0	95.0	+33.0		
27	M	2	62.9	90.3	+27.4	113.6	(1.35)
29	F	2	61.4	83.5	+22.1		
31	M	2	71.2	102.3	+31.1		
20	M	3	74.4	91.4	+17.0		
21	M	3	71.5	97.6	+26.1		
24	M	3	67.7	98.8	+31.1	99.4	(1.18)
28	F	3	64.4	89.6	+25.2		

diet, two groups of litter A were again fed the supplemented basal diets (diets 2 and 3). Growth was resumed, although, as anticipated, the rate was not as great with these now heavier animals.

Two of the rats of group 3 of litter B were placed on diet 4 after the initial period on diet 3. This diet resembled diet 1 but contained gluconolactone instead of glucose. Glucono-

TABLE 3

Changes in weight of rats on low-caloric diets and diets supplemented with glucose and gluconolactone.

The values presented represent changes in weight on the various diets indicated. The periods are of 2 weeks' duration, except for period I with litter B, which extended for 3 weeks.

RAT NO.	PERIOD I		PERIOD II		PERIOD III	
	Diet	Weight change	Diet	Weight change	Diet	Weight change
		gm		*gm.*		*gm.*
			Litter A			
11	1	+ 4.7	3	+21.0		
16	1	+ 7.6	3	+13.4		
18	1	+15.2	3	+16.5		
12	2	+16.3	1	+ 8.1	3	+13.3
13	2	+15.1	1	— 3.8	3	+14.2
19	2	+25.3	1	— 2.4	3	+13.0
14	3	+17.4	1	— 3.2	2	+ 9.9
15	3	+25.2	1	— 0.1	2	+12.4
17	3	+15.7	1	— 5.5	2	+10.1
			Litter B [1]			
22	1	+11.8	3	+19.8		
23	1	+ 7.1	3	+13.5		
25	1	+15.9	3	+ 6.2		
30	1	+17.9	3	+15.2		
26	2	+33.0	1	— 2.6		
27	2	+27.4	1	— 0.7		
29	2	+22.1	1	+ 3.2		
31	2	+31.1	1	+ 2.2		
20	3	+17.0	2	+14.1		
21	3	+26.1	4 [2]	— 3.6		
24	3	+31.1	4 [2]	— 7.1		
28	3	+25.2	1	— 4.8		

[1] Period I is of 3 weeks' duration and period II of 2 weeks' duration, in the experiments with litter B.

[2] Diet 4 is the same as diet 1 except that 1.5 gm. of the gluconolactone replaces the glucose (1.5 gm.). The caloric values of diets 1 and 4 are essentially the same.

lactone was the only available "carbohydrate" except for the carbohydrate present in the yeast tablet. These two animals showed, as expected, prompt loss of weight, although in one case the loss of weight was no greater than the loss of an animal of the same group which was fed the basal diet (rat 28).

We believe that the data here presented and similar data obtained by us indicate clearly that in small quantities the gluconolactone can be utilized for growth almost as efficiently as glucose. Since the limiting factor in the basal diets was the low caloric value, and since the addition of glucose or the gluconolactone increased the rate of growth significantly, it seems logical to conclude that the gluconolactone served as a source of energy either by conversion to glucose and oxidation of the glucose thus formed, or by direct oxidation. Experiments are planned to test further the fate of the gluconolactone in the animal organism. The value of gluconolactone in nutrition would seem to be limited definitely by its laxative action in the gastrointestinal tract (Mitchell, Cook and O'Brien, '39).

SUMMARY

Young white rats were fed a diet whose limiting factor was an inadequate caloric value. When this basal diet was supplemented by either glucose or d-glucono-δ-lactone as a source of additional calories, growth at a rate definitely greater than that of animals fed the basal low-caloric diet was observed. Glucose and the gluconolactone were almost equally effective in the promotion of growth. These studies are believed to indicate that under the conditions of the present experiments, gluconolactone in limited amounts may serve as a source of energy, utilization being effected either by direct oxidation or by conversion to glucose and oxidation of the latter.

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THE EFFECT OF COMMERCIAL CLARIFICATION ON THE VITAMIN CONTENT OF HONEY [1] [2]

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In a previous paper (Haydak and co-workers, '42) we reported the vitamin content of clarified and unclarified honeys. It subsequently developed that the clarification was done under laboratory conditions and the question was raised whether commercial processing of this type would give the same reductions in vitamin content. In order to obtain data on this question, samples of unclarified and clarified honey were obtained from two commercial packers.

The methods used were: for thiamine, that of Hennessy and Cerecedo ('39); for riboflavin, that of Snell and Strong ('39); for ascorbic acid, Bessey ('38); pantothenic acid Strong, Feeney and Earle ('41), and for nicotinic acid, the method of Melnick and Field ('40). We also determined the anti-hemorrhagic activity of clarified and unclarified honeys, following method of Almquist ('41) as modified slightly by Vivino and co-workers ('43).

The results of the determinations are presented in table 1, in comparison with those previously published. From the table it is evident that there was a general decline in the concentration of vitamins due to the commercial clarification, although generally to a lesser degree than in the laboratory processed sample.

The prothrombin time was 3′ 55″ and 4′ 11″; 4′ 19″ and 4′ 07″ for unclarified and clarified honeys, respectively, when 2 gm. of honey were supplied daily for 4 days during the curative period to vitamin K depleted chicks. This corresponds closely with the average prothrombin time of 4′ 02″ for the positive controls which received 0.5 μg. of synthetic vitamin K (2-methyl-1, 4-naphthoquinone in ethyl laurate) daily during the same period. The prothrombin time of the negative controls was 25 minutes. It is apparent that clarification did not perceptibly affect the antihemorrhagic activity of honey. This fact would suggest that this activity is not due to the pollen present in honey but to substances dissolved in it.

TABLE 1

Vitamin content per 100 gm. of clarified and unclarified honey.

SAMPLE	THIAMINE	RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID	ASCORBIC ACID
	μg.	μg.	μg.	μg.	mg
(a) Unclarified	5.1	80.0	100.0	400.0	2.1
(b) Clarified	3.6	62.0	92.0	290.0	1.9
Decrease %	*30.0*	*22.5*	*8.0*	*27.5*	*9.5*
(a) Unclarified	5.9	61.0	81.0	720.0	2.0
(b) Clarified	4.3	33.0	63.0	610.0	1.6
Decrease %	*27.1*	*45.9*	*22.2*	*15.3*	*20.0*
Previous results, laboratory process					
(a) Unclarified	8.4	93.0	176.0	870.0	2.7
(b) Clarified	5.4	64.0	97.0	460.0	1.9
Decrease %	*35.7*	*31.2*	*44.9*	*47.1*	*29.6*

SUMMARY AND CONCLUSIONS

Two pairs of samples of clarified and unclarified honey were assayed for thiamine, riboflavin, pantothenic acid, nicotinic acid and ascorbic acid contents. There was a general decrease in the vitamin concentration of the clarified samples, ranging from 8 to 45%. There was no perceptible reduction in the antihemorrhagic activity of the clarified honeys compared with the unclarified controls.

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EFFECT OF INCREASING CALCIUM CONTENT OF
· DIET UPON RATE OF GROWTH AND LENGTH
OF LIFE OF UNMATED FEMALES

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In previous work (Sherman and Campbell, '35) the calcium content of a diet which had been proven to be adequate for growth and reproduction generation after generation was increased by the addition of calcium carbonate from the initial level of 0.20% to a level of 0.34% of calcium in the air-dry food. This resulted in (1) a very slightly increased growth; (2) no significant difference in adult size but a higher level of adult vitality; and, (3) in the males, an increased length of life. The effect upon the length of life of the females was, however, somewhat in doubt. The experimental animals (rats) had been mated early and allowed to breed as freely as possible. The females which received the diet containing the larger amount of calcium reared more young and brought them to a higher average weight at a standard weaning age. When, then, these females lived only insignificantly longer than those on the diet of lower calcium content, the question arose whether this was because the females either were less able than the males to make full use of the extra food calcium, or had more ways in which to invest it. Was it because their extra food calcium had been invested in the bearing and suckling of more young that the lives of the mothers were not more distinctly prolonged?

The present paper reports the results of experiments designed to ascertain whether females not called upon to furnish

calcium to offspring would gain in longevity through the same increase of calcium intake.

Equal numbers of female rats of the same hereditary and nutritional background were fed the same diets 16 and 162 as‑ described in the paper above noted. The initial age was 28 days, and each experimental animal was matched by a litter‑ mate sister, the one receiving diet 16 with 0.20%, and the other diet 162 with 0.34%, of calcium. All of these experimental animals were observed daily and weighed weekly throughout the complete life span. The data of early growth and length of life are summarized in table 1.

TABLE 1

Records of unmated female rats on diets of different calcium contents.

CATEGORY OF INTEREST	ON DIET 16. 0 20% CALCIUM		ON DIET 162. 0 34% CALCIUM	
	No of cases	Mean ± P.E [1]	No of cases	Mean ± P E [1]
Gain in weight,				
5th to 8th weeks *(gm.)*	51	46.9 ± 0.83	51	58.4 ± 0.69
Age at death *(days)*	51	795 ± 16.	51	858 ± 12.

[1] This precision measure is the classical probable error of the mean.

DISCUSSION

As in the experiment previously mentioned, growth was slightly more rapid in the animals receiving the higher calcium intake. The noticeable difference was that in the unmated females, the increased calcium intake resulted in an extension of the life cycle by an average of 63 days or 8%, or in essen‑ tially equal proportion to the gain shown by the males in the experiments of the previous series above mentioned. The significance of the increase in length of life is further shown by the fact that the proportion of cases living to 900 days and over was 45% among those of higher, against 31% among those of lower, calcium intake.

These findings gain added interest when considered in connection with those of a related investigation in this laboratory in which it was found that mated females receiving food of still higher calcium content both raised more young and enjoyed an increased length of life (Van Duyne, Lanford, Toepfer, and Sherman, '41).

CONCLUSION

A limited addition of calcium to a diet of about minimal adequate calcium content results in equally increased length of life in comparable male and female rats if the latter do not invest the extra calcium in increased reproduction and lactation instead.

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FIELD PEAS AS A SOURCE OF PROTEIN FOR GROWTH [1]

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Finks, Jones and Johns ('22) reported that the field pea (Pisum sativum) when fed as 75% of the diet had given "normal growth" in young rats, and the opinion has been very generally held that the protein of the field pea supplies adequate amounts of all the nutritionally essential amino acids. Deficiencies which had occurred in other experiments could always be explained as due to the use of other legumes designated by the common name, pea. Thus Finks and his co-workers had found that cystine was a limiting factor in growth with the cowpea (Vigna sinensis), and McCollum, Simmonds and Parsons ('19) had reported that split peas identified as Vicia sativa, when soaked, autoclaved and dried in air, did not support very good growth if fed as 45% of the diet. Sure ('21) reported that in repeating McCollum's work he encountered conflicting results which could be traced to the inadvertent use of one lot of field peas. More recently Cook and Robertson ('41) have reported that the protein of Alaska field peas when added to a cereal ration for chicks has about 50% of the supplementary value of casein and that dl-valine does not appear to be the limiting factor in the nutritive value of pea protein.

Finks and his co-workers ('22) described their product as a smooth, yellow, split pea. The Alaska field pea, also identified

as Pisum sativum, is a smooth, round pea green in color. In the immature state these peas are used chiefly for canning purposes and when mature are marketed as split peas for human consumption. Dried peas are becoming increasingly valuable as a source of protein for low cost diets. Field peas and their by-products are used extensively in animal feeding. This report deals with the nutritive value of peas, both raw and heat-treated, as the sole source of protein and the effect on growth in young rats by the addition of dl-methionine and of l-cystine to the diet.

EXPERIMENTAL METHOD

Alaska field peas (Pisum sativum), grade no. 1,[2] were cleaned, culled, and ground in a Wiley mill through a 1 mm. sieve. After grinding, the peas were well mixed and divided into three portions. For heat treatment one portion was spread out in layers approximately $\frac{1}{2}$ inch thick and baked in a Freas oven for $1\frac{1}{2}$ hours at 140° C., and another portion was spread out about 1 inch thick and autoclaved for $1\frac{1}{2}$ hours at 17 pounds pressure after which they were dried at room temperature. The third portion was fed in the raw state.

Raw, baked and autoclaved peas were analyzed for moisture and for nitrogen, and the quantity of each required to furnish a protein level (N × 6.25) of 10% was used in making up the diets. A diet containing 10% vitamin-free-casein was used as a standard of comparison in the feeding tests. The composition of the diets is presented in table 1.

The added amino acids, dl-methionine and l-cystine, when used were thoroughly mixed with the diets, replacing an equivalent amount of sugar without adjustment in the nitrogen of the diet.

Each rat received as a supplement six times a week pure vitamins [3] as follows: thiamine hydrochloride 20 μg., ribo-

flavin 20 µg., pyridoxine 20 µg., calcium pantothenate 80 µg., nicotinic acid 250 µg., choline hydrochloride 10 mg.

The rats were 21 or 22 days old when started on the test diets. They were housed in individual cages and weighed weekly for 8 weeks. Food consumption records were made at each weighing.

The statistical analyses [4] of the data were made according to the methods of Snedecor ('40).

<div align="center">TABLE 1</div>

Composition of diets used in pea protein studies.

INGREDIENT	CASEIN		PROTEIN IN PEAS			
			Raw		Baked	Autoclaved
	10%	18%	10%	20%	10%	10%
	gm.	gm.	gm.	gm.	gm.	gm.
Raw peas	...	..	438	876	...	...
Baked peas	...	...	...	...	405	...
Autoclaved peas	..	..	...	...	...	463
Casein [1]	100	180	...	...	...	...
Salt mixture [2]	40	40	40	40	40	40
Cottonseed oil [3]	30	30	30	30	30	30
Cod liver oil	20	20	20	20	20	20
Cane sugar	790	690	472	34	505	447
Agar	20	40	...	...	...	...
	1,000	1,000	1,000	1,000	1,000	1,000

[1] Labco.

[2] Osborne and Mendel, 1919, J. Biol. Chem., vol. 37, p. 572.

[3] Wesson.

<div align="center">RESULTS AND DISCUSSION</div>

The results of this series of experiments are summarized in tables 2 and 3. It is evident that the lack of available methionine is the principal growth-limiting deficiency of raw peas (lots 3, 4, 5, 6). The rates of gain were approximately four times greater in a period of 8 weeks when raw peas were supplemented with 0.3 to 0.9% methionine. Furthermore, rats fed on peas plus 0.3% methionine gained 47% more in 8 weeks than those receiving casein at the same protein level, and re-

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[4] Credit is due M. L. Buchanan, Department of Animal Husbandry, for valuable assistance in statistical treatment of data.

TABLE 2

Average growth response by rats fed diets used in the investigation of pea protein.

LOT NO.	DIET	NUMBER OF RATS	AVERAGE GAIN IN 8 WEEKS (gm.)	AVERAGE GAIN PER GRAM OF PROTEIN (gm.)	AVERAGE FOOD REQUIRED PER GRAM OF GAIN	AVERAGE FOOD CONSUMED DAILY PER RAT
1	10% casein	21	85.2 ± 7.2[1]	1.74 ± 0.03[1]	5.65 ± 0.12[1]	8.49 ± 0.62[1]
2	18% casein	3	151.3 ± 0.4	1.66 ± 0.45	3.36 ± 0.05	9.07 ± 0.14
3	10% raw pea protein	22	29.2 ± 1.6	1.05 ± 0.05	9.96 ± 0.54	4.89 ± 0.10
4	10% raw pea protein plus 0.3% methionine	7	124.7 ± 7.3	2.35 ± 0.05	4.22 ± 0.12	9.34 ± 0.42
5	10% raw pea protein plus 0.6% methionine	12	123.4 ± 5.8	2.58 ± 0.04	4.24 ± 0.49	8.52 ± 0.35
6	10% raw pea protein plus 0.9% methionine	5	107.2 ± 11.9	2.36 ± 0.10	4.28 ± 0.20	8.02 ± 0.57
7	10% raw pea protein plus 0.5% cystine	7	35.4 ± 2.0	1.35 ± 0.05	7.50 ± 0.27	4.69 ± 0.12
8	20% raw pea protein	3	86.3 ± 3.5	1.00 ± 0.06	5.01 ± 0.33	7.70 ± 0.41
9	10% baked pea protein	10	20.1 ± 2.0	0.78 ± 0.05	13.30 ± 1.08	4.48 ± 0.17
10	10% autoclaved pea protein	15	15.1 ± 1.0	0.59 ± 0.03	18.10 ± 1.25	4.59 ± 0.12
11	10% autoclaved pea protein plus 0.6% methionine	6	118.1 ± 5.8	2.35 ± 0.05	4.27 ± 0.01	8.59 ± 0.24
12	10% autoclaved pea protein plus 0.5% cystine	3	30.0 ± 1.5	1.16 ± 0.01	8.20 ± 0.08	4.59 ± 0.17

[1] Standard error.

TABLE 3

Statistical comparisons of growth on various diets.

LOT NO.	DIETS COMPARED	GRAMS GAIN IN 8 WEEKS			GRAMS GAIN PER GRAM PROTEIN		
		Mean difference	Standard error of difference	t[1]	Mean difference	Standard error of difference	t[1]
1–3	10% casein vs. 10% raw pea protein	56.0	± 7.3	7.67	0.69	± 0.06	11.50
3–10	10% raw pea protein vs. 10% autoclaved pea protein	14.1	± 1.8	7.83	0.46	± 0.06	7.60
3–9	10% raw pea protein vs. 10% baked pea protein	9.1	± 2.5	3.64	0.27	± 0.07	3.80
3–4	10% raw pea protein vs. 10% raw pea protein plus 0.3% methionine	95.5	± 7.4	12.91	1.30	± 0.07	18.50
3–5	10% raw pea protein vs. 10% raw pea protein plus 0.6% methionine	94.2	± 6.0	15.70	1.53	± 0.06	25.50
3–6	10% raw pea protein vs. 10% raw pea protein plus 0.9% methionine	78.0	± 12.0	6.50	1.31	± 0.11	11.91
3–7	10% raw pea protein vs. 10% raw pea protein plus 0.5% cystine	6.2	± 2.5	2.48	0.30	± 0.07	4.20
10–11	10% autoclaved pea protein vs. 10% autoclaved pea protein plus 0.6% methionine	103.0	± 5.9	17.40	1.86	± 0.06	31.00
10–12	10% autoclaved pea protein vs. 10% autoclaved pea protein plus 0.5% cystine	14.9	± 1.5	9.93	0.57	± 0.03	19.00
2–8	18% casein vs. 20% raw pea protein	97.9	± 6.0	16.32	1.30	± 0.07	18.57
4–5	10% raw pea protein plus 0.3% methionine vs. 10% pea protein plus 0.6% methionine	1.3	± 9.3	0.14	0.23	± 0.06	3.83
5–6	10% raw pea protein plus 0.6% methionine vs. 10% pea protein plus 0.9% methionine	16.2	± 13.2	1.23	0.22	± 0.10	2.20

[1] A t value of 3.707 establishes the 1% point for the comparisons based on the smallest number of degrees of freedom; therefore a value of 3.707 or larger is highly significant.

quired 25% less food per gram of gain (lots 1, 4). Supplementing the raw pea diet with 0.3% or more methionine practically doubled the food consumed daily (lots 3, 4, 5, 6) and decreased the amount of food required per gram of gain by one-half.

The most efficient level of supplementary methionine for 10% raw pea protein appears to be not more than 0.3% of the diet. Levels of 0.6 and 0.9% caused no increase in rate of gain or in average gain per gram of protein over that with 0.3%.

The results given in table 2 show that the baking and autoclaving of field peas decreased its value for growth of rats when compared with raw peas. The rate of gain observed on autoclaved peas was one-half as much as on raw peas (lots 3, 10) and baking decreased the gains but not as greatly (lots 3, 9). Apparently some nutritional factor associated with protein adequacy is destroyed by both dry and moist heat. Field peas react differently to heat treatment than do soybeans, for Hayward, Steenbock and Bohstedt ('36) reported improvement in the nutritive value of soybean protein when subjected to heat treatment. According to Hayward and Hafner ('41), autoclaving soybeans possibly increases the availability of cystine and methionine as well as other essential amino acids. Morgan ('31), however, found that dry heating damaged cereal proteins and that the injurious effect could be corrected by feeding histidine or lysine with the heated protein. Autoclaving peas seems to destroy cystine instead of making it more available. The evidence for this conclusion is the fact that the addition of 0.5% cystine to the autoclaved pea diet gave a significant increase in rat growth (lots 10, 12, table 3), while supplementing raw peas with 0.5% cystine gave no significant increase in rate of growth (lots 3, 7, table 3). Since the growth rate on a diet of autoclaved peas and 0.5% cystine is the same as the growth obtained on raw peas, it is concluded that moist heat destroys cystine in peas. In a protein study with canned garden peas which had been cooked, Mitchell and Beadles ('30) showed that the addition of cystine to the diet significantly increased the rate of growth of rats.

The rats on the raw, baked and autoclaved pea diets ingested approximately the same amount of food daily, 4.89, 4.48, and 4.59 gm., respectively, while there was a marked decrease in the efficiency of food utilization by the rats fed heat treated peas. The average food required per gram of gain was, respectively, 9.96, 13.30 and 18.10 gm. These data clearly indicate that the differences obtained showing a decreasing effect of heat on the nutritive value of peas were not due to a lowered food intake. Instead, they suggest that either baking or autoclaving tends to destroy some factor, possibly cystine or methionine, which contributes to the more efficient utilization of the diet. The fact that adding 0.5% cystine to autoclaved peas made them equal in growth-promoting properties to raw peas and that the addition of methionine alone produced good growth substantiates the hypothesis that heat treatment, rather than altering the digestibility, destroys a specific nutritional factor, probably cystine.

The addition of 0.6% methionine to the autoclaved pea diet gave a growth response approximately equivalent to raw peas plus methionine, revealing that the loss due to autoclaving may be replaced with methionine. That cystine in animal tissues can be derived from methionine in the diet has been shown by Dawbarn ('38), Beach and White ('39), and Rose and Wood ('41) by measuring the increment of tissue cystine, especially hair, when methionine supplied the dietary sulfur, and by Tarver and Schmidt ('39) who recovered radioactive sulfur from cystine in rat tissue after feeding methionine containing S^{35}.

Mitchell ('38) explains the supplementing effect of cystine to a low methionine diet as due to the need of the animal for both amino acids, the cystine portion of which can be met by cystine itself or can be synthesized by the animal from methionine.

If the diets are examined on the basis of gain per gram of protein eaten, it is evident that raw pea protein at 1.05 compares more favorably with casein at 1.74 than it does when compared on actual gains. The addition of cystine (1.35) im-

proved the efficiency of raw peas somewhat, and methionine (2.35) raised it well beyond the casein value (1.74). Autoclaving the peas reduced the gain per gram of protein from 1.05 to 0.59 while supplementing the autoclaved peas with methionine increased the efficiency to 2.35 per gram of protein. On the same basis the cystine addition increased the efficiency of the autoclaved peas to 1.16, somewhat above the value for raw peas only, but not quite equal to raw peas plus cystine.

Rats fed a diet of raw peas at a 20% protein level made gains comparable to those receiving 10% casein but not what could be called normal with present-day standards, and the gain per gram of protein was essentially the same as for the 10% pea protein diet.

Notwithstanding the apparently good growth obtained from split peas at a 20% protein level by Finks, Jones and Johns, they report that for a 10-week period the gain per gram of protein eaten was only 1.01. This is approximately the efficiency of the Alaska pea for the 8-week test in this investigation (table 2), whether fed to give 10 or 20% protein diets.

In order to check the adequacy of choline, liver fat analyses for a few rats were determined. The fat values were as follows: On the 10% casein diet, 4.0% (average of six animals); raw pea diet, 2.8% (three animals); raw peas plus cystine, 2.1% (three animals); and raw peas with methionine, 2.8%. The animals on the stock diet used in this laboratory show around 2.3% fat in the liver at this age. These data indicate that the added methionine was not needed to supply labile methyl groups for the synthesis of choline.

SUMMARY

1. Raw and heated Alaska field peas (Pisum sativum) have been studied as the sole source of protein in diets for growing rats.

2. The principal growth-limiting deficiency in raw pea protein is methionine.

3. Baking and autoclaving field peas decreased the growth-promoting properties of the protein but did not change the food intake.

4. The addition of cystine to the autoclaved peas permitted a rate of growth comparable to that with raw peas.

5. Rats fed raw pea protein (10% level) plus 0.3 methionine as the sole source of protein made 47% more gain and required 25% less food per unit gain, than rats fed casein at a 10% protein level.

6. Apparently the Alaska field pea is an excellent source of the amino acids essential for growth, with the single exception of methionine.

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THE RATIO OF ASCORBIC ACID, RIBOFLAVIN AND THIAMINE IN RAW AND PASTEURIZED MILK [1]

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Milk has long been recognized as possessing unique nutritive value. It contains protein of high biological value, minerals required for the growth and maintenance of the body, and many of the vitamins essential for human well-being. Because of the importance of the latter, many studies have been made of the vitamin content of cows' milk and the literature contains considerable data concerning the ascorbic acid, riboflavin and thiamine contents of milk produced in many localities under a variety of conditions. However, the extensive literature which was consulted did not contain a single assay of a milk for all three vitamins, ascorbic acid, riboflavin, and thiamine. Accordingly, it seemed desirable to determine the amount of these vitamins present, before and after controlled pasteurization of milk produced under standardized conditions.

EXPERIMENTAL

Source of milk

As a matter of convenience, winter milk (Jan., Feb., and March) was used for this study. It was produced by the college herd of sixty-seven cows which consisted of eighteen Ayrshires, thirteen Guernseys, eighteen Holsteins, eleven Jerseys, and seven Shorthorns. The animals were all normal, in a healthy condition, and under constant veterinary supervision.

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[1] Contribution no. 478, Massachusetts Agricultural Experiment Station.

The stage of lactation, computed as of the middle of the experimental period, averaged 120 days for the Ayrshires, 134 days for the Guernseys, 133 days for the Holsteins, 142 days for the Jerseys, and 115 days for the Shorthorns, or for the herd as a whole, 129 days.

The daily ration of the cows consisted of 6 lbs. of locally grown mixed hay, 18 lbs. of corn silage, 15 lbs. of grass silage, 3½ lbs. of beet pulp, and 9 lbs. of grain containing 14.5% of protein. Because of war restrictions, the ration was lower than normal in concentrates. However, the cows produced an average of 15 quarts of 4% butterfat milk per day.

Pasteurization

The milk from the 3:30 P.M. milkings was promptly cooled and held at 40° F. until the following morning. After the milk obtained from the 3:30 A.M. milking had been cooled, the two were combined and taken to the pasteurizer. Hence, the combined milk was about 10 hours old when it was pasteurized. When the milk had been thoroughly mixed in the pasteurizer, a sample of "raw" milk was taken for analysis. Thus, the studies reported below were conducted upon the total day's production. The milk was pasteurized by the so-called holding process in an 800-quart stainless steel vat pasteurizer.

The time required for the preliminary heating of the milk varied from 27 to 98 and averaged 48 minutes. The temperature of pasteurization was 144° F. and the duration was from 30 to 37 (average 33) minutes.

As soon as pasteurization was completed, the milk was rapidly cooled to 40° F. A sample of pasteurized milk typical of the 800 quarts was taken for analysis and assays for riboflavin and ascorbic acid were commenced immediately but the thiamine assays were not started until 5 hours or so later.

Assay procedures

The ascorbic acid content of the milk was determined by the procedure reported by Holmes, Tripp, Woelffer and Satterfield

('39) and Tripp, Satterfield and Holmes ('37), which was adapted from the 2–6 dichlorophenolindophenol method of Bessey and King ('33).

The riboflavin assay procedure used in this study was a modification of methods suggested by Hand ('39), Hodson and Morris ('39), and Kemmerer ('41).

Twenty-five cubic centimeters of milk were added slowly to 50 cc. of acetone U.S.P. and filtered. A 35 cc. aliquot of the filtrate was diluted with distilled water, and stannous chloride and sodium hyposulphite were added to reduce all of the materials which produce fluorescence. The mixture was made up to 250 cc. and the riboflavin was re-oxidized by thorough agitation with air. Subsequently three 50 cc. aliquots were transferred to 125 cc. Erlenmeyer flasks. One cubic centimeter of standard riboflavin solution was added to aliquot ''b'' which served as a positive control. Aliquot ''c'' was reduced with 1 cc. of sodium hyposulphite solution and served as a negative control, which contained all substances, except riboflavin, present in aliquot ''a'' (the sample) and aliquot ''b''. The cubic centimeters of each aliquot were transferred to the cuvettes for fluorometric assay. The assays were made with a spectrophotometer [2] adapted for making fluorescence measurements. By reading the negative control ''c'' both before and after sample ''a'' and aliquot ''b'', it was possible to detect instantly any changes in the electric current, the setting of the instrument, or other mechanical factors which would affect the accuracy of the reading. Such constant checking of the instrument is not possible when one relies upon a reference curve for interpreting the reading of the sample.

The readings for ''a'', ''b'', and ''c'' supply data for computing the riboflavin content of the sample ''a''.

Since riboflavin may be destroyed by light (Hogan and Hunter, '28; Supplee, Ansbacher and Bender, '35; György, '35; and others) amber glassware was used and the assays were made in a specially prepared semi-basement laboratory

[2] Coleman Universal Spectrophotometer.

from which all daylight was excluded. The 100-watt Mazda bulbs were surrounded with long metal cylinders that prevented direct light reaching the laboratory tables at the side of the room. Meter measurements of the light intensity at the site of the precipitation, filtration and other assay procedures showed the light to be from one to two candle power.

The thiamine content of the milk was determined by a modification of the thiochrome method of Hennessy and Cerecedo ('39).

<div align="center">RESULTS</div>

The ascorbic acid content of the raw milk (table 1) varied from 14.0 to 22.5 mg. per liter, with an average of 19.7 $\pm$ 1.8 mg. per liter. After the milk had been pasteurized its ascorbic acid content varied from 7.0 to 19.1 mg. per liter and averaged 15.9 $\pm$ 2.7 mg. per liter. The change in the ascorbic acid value of the milk from 19.7 mg. to 15.9 mg. per liter represents an 18.3% loss caused by pasteurization. This result is practically identical with the 18.71% loss reported in an earlier investigation by Holmes, Tripp, Woelffer and Satterfield ('39) in which herd milk from 175 Guernsey, Holstein, and Ayrshire cows was used. The 5 months' experimental period began in mid-August. The same assay procedure was followed, and the equipment was of the same type, namely, commercial size, stainless steel, spray vat equipped with stainless steel agitators that kept the milk gently in motion during the holding period which was for 30 minutes at 144° F. However, the potency of the milk was different for the two studies. In the earlier study, the values were 17.26 and 14.03 mg. per liter for the raw and pasteurized certified milk, whereas in the present study the values were 19.7 and 15.9 mg. of ascorbic acid per liter, respectively, for the raw and pasteurized milk. Thus, the results obtained in the two studies indicate very strongly that approximately one-fifth of the ascorbic acid content of fresh whole milk is lost during pasteurization in stainless steel equipment for 30 minutes at 143°–144° F.

Referring to table 1, it will be noted that the riboflavin content of the thirty-one samples of raw milk varied from 1.35 to

TABLE 1

The ascorbic acid, riboflavin and thiamine content of milk.

SAMPLE NO.	ASCORBIC ACID		RIBOFLAVIN		THIAMINE	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
	mg. /liter		*mg. /liter*		*mg. /liter*	
1	21.0	15.0	1.41	1.42	0.33	0.31
2	20.5	15.7	1.52	1.51	0.35	0.29
3	19.6	14.9	1.42	1.42	...	..
4	19.0	14.5	1.56	1.54	0.33	0.29
5	19.6	15.0	1.47	1.37	0.35	0.31
6	17.6	12.6	1.53	1.48	0.32	0.32
7	21.5	17.7	1.71	1.67	0.32	0.32
8	21.5	17.7	1.56	1.52	0.33	0.29
9	22.5	8.8	1.52	1.42	0.32	0.26
10	20.1	15.1	1.55	1.52	0.33	0.32
11	20.0	15.0	1.56	1.50	0.34	0.32
12	21.4	17.4	1.75	1.67	0.33	0.30
13	14.0	7.0	1.58	1.49	0.32	0.30
14	18.7	14.1	1.39	1.47	0.30	0.28
15	20.8	18.0	1.58	1.69	0.32	0.30
16	18.2	14.6	1.50	1.49	0.35	0.33
17	19.2	16.3	1.56	1.48	0.35	0.32
18	20.5	17.0	1.49	1.40	...	0.21
19	20.2	17.1	1.35	1.45	0.33	0.31
20	20.1	16.6	1.44	1.19	0.34	0.30
21	22.1	19.1	1.49	1.34	0.33	0.31
22	19.9	18.7	1.43	1.43	0.34	0.32
23	16.0	18.1	1.55	1.48	0.34	0.32
24	18.5	18.5	1.72	2.06	0.34	0.24
25	20.9	19.0	..	...	0.34	0.31
26	19.2	15.3	1.42	1.34	0.31	0.29
27	20.7	17.7	1.38	1.45	0.29	0.29
28	19.6	17.2	1.44	1.33	0.31	0.30
29	20.3	17.2	1.48	1.46	0.35	0.32
30	20.1	17.4	1.54	1.42	...	0.34
31	20.6	18.2	1.48	1.36	0.32	0.31
32	17.0	13.6	1.52	1.60	0.33	0.32
Average	19.7	15.9	1.51	1.48	0.33	0.30
	± 1.8 [1]	± 2.7	± 0.09	± 0.01	± 0.02	± 0.03

[1] Standard deviation.

1.75 mg. per liter with an average of 1.51 ± 0.09 mg. per liter. The amount of riboflavin in the pasteurized milk varied from a minimum of 1.19 mg. to a maximum of 2.06 mg. per liter, with an average of 1.48 ± 0.01 mg. per liter. Thus in these experiments the pasteurizing process caused a loss of approximately 2% of the riboflavin. This result is in very good agreement with those of Javillier ('40), who reported that preheating and sterilizing milk caused no loss of riboflavin, and those of Houston, Kon and Thompson ('40), who found the riboflavin content of February, June, and July milk to be slightly increased after both pasteurization and sterilization.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. per liter with an average value of 0.33 ± 0.02 mg. per liter. After pasteurization the thiamine content varied from a minimum of 0.21 mg. to a maximum of 0.34 mg. per liter and the average value for the thirty-one samples was 0.30 ± 0.03 mg. per liter of milk. Thus 9.1% of thiamine was lost during pasteurization of the milk by the holding process. This value is much less than that reported by a number of other investigators. Elvehjem ('41) reported that pasteurization caused a 20% loss of thiamine. Weckel ('36) found that commercial sterilization destroys approximately one-third of the vitamin B_1 in milk; and Houston, Kon and Thompson ('40) state that up to 50% of vitamin B_1 is destroyed in the course of heat treatment.

The ratio of ascorbic acid, riboflavin and thiamine in raw and pasteurized milk has been computed to show the relation of the riboflavin content to those of thiamine and of ascorbic acid, and the relation of ascorbic acid to thiamine.

The ratio of ascorbic acid to riboflavin in the raw milk varied from a minimum of 8.9 for sample 13 to a maximum of 15.0 for samples 19 and 27. Considering the group of samples as a unit, the average ascorbic acid content of the raw milk was 13.1 ± 1.43 times that of the riboflavin. The ratio of ascorbic acid to riboflavin in the pasteurized milk was somewhat lower than for the raw milk because of the loss of about one-fifth of the ascorbic acid during the pasteurization process. The mini-

mum value was 4.7 for sample 13 and the maximum was 14.3 for sample 21. On the average, the ascorbic acid content of the pasteurized milk was 11.0 ± 1.93 times its riboflavin content.

The ratio of the riboflavin to the thiamine content of the raw milk varied from sample 19 in which the riboflavin content was 4.1 times that of thiamine to sample 7 and 12 which contained 5.3 times as much riboflavin as thiamine. On the average the raw milk contained 4.6 ± 0.3 times as much riboflavin as thiamine, and the riboflavin content of the pasteurized milk averaged 4.9 ± 0.81 times that of thiamine.

The ratio of the ascorbic acid to the thiamine of raw milk was much higher than the ratio of riboflavin to thiamine or of ascorbic acid to riboflavin. The ratio of ascorbic acid to thiamine in raw milk ranged from 43.8 for sample 13 to 71.4 for sample 27 and averaged 59.8 ± 6.40. There was a larger loss of ascorbic acid than of thiamine during the pasteurization process, and the ratio ranged from 23.3 for sample 13 to 77.1 for sample 24. The average ascorbic content of the pasteurized milk was 53.8 ± 9.63 times that of the thiamine.

SUMMARY

The ratios to each other of ascorbic acid, riboflavin, and thiamine have been determined for winter milk produced under controlled conditions by the college herd of Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows. The vitamin contents of the milk were determined for samples taken just previous to and immediately following pasteurization by the holding process for 30 minutes at 143°–145° F.

The ascorbic acid content of the raw milk ranged from 14.0 mg. to 22.5 mg. and averaged 19.7 ± 0.18 mg. per liter; after pasteurization in stainless steel equipment, the extreme values were 7.0 mg. and 19.1 mg., with an average value of 15.9 ± 2.7 mg. per liter.

The riboflavin content of the raw milk varied from 1.35 mg. to 1.75 mg. and averaged 1.51 ± 0.09 mg. per liter. The corresponding riboflavin values for the pasteurized milk were 1.19, 2.06, and 1.48 ± 0.01 mg. per liter, respectively.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. and averaged 0.33 ± 0.02 mg. per liter. Corresponding thiamine values for the pasteurized milk were 0.21, 0.34, and 0.30 ± 0.03 mg., respectively.

The ratios of riboflavin, ascorbic acid, and thiamine were computed for both the raw and the pasteurized milk. The raw milk contained 4.6 ± 0.3 times as much riboflavin as thiamine, 13.1 ± 1.43 times as much ascorbic acid as riboflavin, and 59.8 ± 6.40 times as much ascorbic acid as thiamine. On the average, the pasteurized milk contained 4.9 ± 0.81 times as much riboflavin as thiamine, 11.0 ± 1.93 times as much ascorbic acid as riboflavin, and 53.8 ± 9.63 times as much ascorbic acid as thiamine.

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SOME RESULTS OF FEEDING RATS A HUMAN DIET LOW IN THIAMINE AND RIBOFLAVIN

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A report of some of our results of feeding rats a human diet low in thiamine has been made (Higgins, Williams and Mason, '43). The present report covers the results obtained during a similar study wherein young rats of the same genetic strain were fed a human diet low in both thiamine and riboflavin.

The riboflavin content of the basal diet in our initial study was 2.5 µg. per gram. In order to reduce this level, certain changes were made in some of the constituents of the original basal diet. Skim milk powder, cheese and skim milk were omitted altogether. Larger portions of bread, beef roast and butter were allowed and cream was added. Except for these changes and the substitution of certain fruits and cereals the basal diet resembled that used before. Clinical results of providing this diet to human subjects have been reported (Williams, Mason, Cusick and Wilder, '43). The components of this basal diet are shown in table 1. Vitamins A and D, ferrous sulphate and tricalcium phosphate were added to the diet in amounts sufficient to provide not less than 10 International Units (I. U.) of vitamin A, 1.0 I. U. of vitamin D, 0.4 mg. of ferrous sulfate and 1.2 mg. of tricalcium phosphate per gram of diet.

The five diets tested are designated in this report as diets E, F, G, H and J. They differed from one another only in the flour from which the bread component of each diet was prepared. In diet E (table 1) the bread was prepared with a patent white flour; in diet F, the patent white flour was restored in content of thiamine to the approximate level of

TABLE 1

Biochemical data on diets used in experiment.

ANALYSIS OF DIET E	Carbohydrate	(%):	68.6
	Protein	(%):	13.1
	Fat	(%):	16.5
	Calories per gram·		4 7
	Calories from carbohydrate	(%):	56.1
	Calories from protein	(%):	13.3
	Calories from fat	(%):	30.6

	Per gram of dry diet	
DIET	Thiamine	Riboflavin
	µg.	*µg.*
E — Basal diet	0.96	2.00
F — Basal diet supplemented with thiamine	1.62	2.00
G — Basal diet suppplemented with thiamine and riboflavin	1.82	2.90
H — Basal diet supplemented with thiamine, riboflavin and niacin [1]	1.78	2.90
J — Basal diet (bread component of whole wheat)	1.94	2.60

[1] 6 mg. of niacin in each pound of flour.

whole wheat flour; in diet G, the patent white flour was restored in content of thiamine and riboflavin to the approximate levels of whole wheat flour; in diet H, the flour was identical with that of diet G except that niacin was added in amounts equal to 6 mg. per pound (0.5 kg.) of flour; and in diet J the bread was prepared with whole wheat flour. Each diet when thoroughly mixed weighed 1,087.8 gm. and, when

dried at a temperature of 65° C. for consumption by rats, weighed 465 gm. When ready for use, assays were made for the thiamine and riboflavin contents of the dried diets. We used the methods of Hennessy ('41) and of Conner and Straub ('41) for these determinations. The results of these assays are summarized in table 1.

Young rats, weighing about 80 gm., were used for this study. They were housed in metal cages with a screen bottom, four to a cage; food and water were always available. Eight animals were fed each of the diets tested and observations were continued for 14 weeks. All animals were weighed each week. Beginning at the end of the eleventh week, and for 10 days threafter, the average intake of food and water per day was recorded. Diets were provided in containers, all waste was salvaged and the change of weight was recorded as food consumed. Likewise water, provided in bottles attached to the cages, was measured daily and the decreases of volume were recorded as cubic centimeters consumed.

At the end of the twelfth week, samples of heart blood were withdrawn for study of the number and size of the erythrocytes, the concentration of hemoglobin, the percentages of reticulocytes and the number of leukocytes. At the end of the experiment (14 weeks), all animals were killed and portions of the livers, thyroids and pituitaries were fixed in Zenker-formalin solution and appropriately prepared for histologic study. Analyses of the concentrations of thiamine and riboflavin per gram of tissue were made of the livers, kidneys, skeletal muscles and testes of five animals which had eaten the basal ration for 14 weeks. These data were contrasted with analyses made of these tissues from five animals which had eaten our standard laboratory ration for a comparable period.

RESULTS

Body weight. Marked differences in the growth rates of these groups of rats are recorded in figure 1. These weight data confirmed our earlier observations that fortification

of flour with thiamine had only a slight influence on the growth rate. Fortification with thiamine and riboflavin improved the diet sufficiently to promote greatly increased growth rates. The addition of niacin was of only slight value and the conclusion is indicated that fortification of the flour with these three vitamins did not promote growth rates nearly as satisfactorily as the substitution of whole wheat flour for the patent flour.
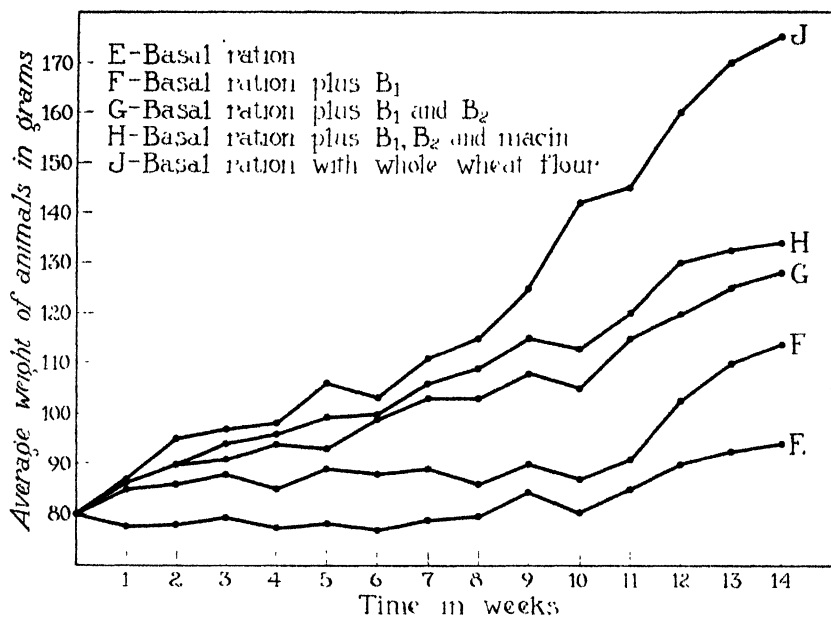
Fig. 1   Growth curves of five groups of rats fed the human diets in which the only variable was the flour from which the bread component was made.

Intake of food and water. The data on intake of food and water over a 10-day period near the close of the experiment are assembled into table 2. They show that rats eating the basal diet were taking 5.7 gm. of food (26.8 cal.) per day and yet their weights remained essentially constant. When the thiamine was added, the intake of food increased to an average of 40.0 cal. per day and yet the average gain was less than 1 gm. a day. Even animals eating diet J, consuming

42.7 cal. per day, gained on the average only 1 gm. a day during the test period. When the food intake per unit of surface area (table 2) was computed, animals eating diet J were taking less food than those eating diet H and yet their body weights were considerably greater.

Although appetites were apparently good and the caloric intake was adequate to promote better growth, effective utilization of the foods apparently was not obtained in any of

TABLE 2

The intake of food and water over a 10-day period and the blood findings.

CATEGORY OF INTEREST	DIET				
	E	F	G	H	J
Food intake: gm./rat/day	5.7	8.5	7.7	9.6	9.1
Food intake: gm./100 sq. cm. surface area/rat /day	3.1	4.5	3.5	4.3	3.6
Water intake: cc./rat/day	7.7	9.0	11.3	11.0	13.9
Blood — Erythrocytes: millions/cu. mm.	7.46 ± 0.19 [1]	7.81 ± 0.24	8.76 ± 0.16		8.85 ± 0.11
Blood — Hemoglobin: gm./100 cc.	12.5 ± 0.34	13.5 ± 0.21	14.0 ± 0.14		15.1 ± 0.30
Blood — Reticulocytes: %	3.0 ± 0.27	2.9 ± 0.38	1.5 ± 0.16		0.9 ± 0.09
Blood — Leukcoytes: thousands/cu. mm.	9.3 ± 0.89	10.9 ± 0.65	10.17 ± 0.47		17.03 ± 1.21

[1] Standard error.

the groups. This may be due to the low riboflavin content of all the diets which were tested, even diet J (2.6 µg. per gram). These rats, consuming on the average 9.1 gm. of food per day, were getting but 23.6 µg. of riboflavin daily. In our initial study, very much better growth than in the present study was obtained among animals eating the ration containing whole wheat flour, wherein the intake of riboflavin was about 35.0 µg. daily.

Observations on the blood. The data assembled from blood samples at the end of the twelfth week are condensed in table 2. The means of the determinations together with their standard errors are included. Hypochromic anemia developed in animals which ate diet E. The addition of thiamine alone improved the erythrocyte and hemoglobin levels somewhat, although not significantly so, but the addition of both thiamine and riboflavin stimulated production of erythrocytes, resulting in an average increase of 1.30 ± 0.24 million cells per cubic millimeter of blood. This difference is more than five times the standard error. Likewise the concentration of hemoglobin per 100 cc. of blood increased by 1.5 ± 0.36 gm. when animals ate diet G. Diet J, which contained whole wheat bread, did not increase the number of erythrocytes, but did significantly increase the homoglobin levels over those of diet G. These data confirm our earlier observation, as well as those of others, that the addition of riboflavin, as in diet G, stimulated production of erythrocytes and hemoglobin.

The liver. The livers of all animals which ate diet E showed extensive peripheral degeneration throughout most of each hepatic lobule (fig. 2). The cells were necrotic; they contained extensive vacuoles and their nuclei were pyknotic. Appropriate stains revealed varying amounts of fat droplets, indicating, presumably, a fatty degeneration in the peripheral portions of the lobules. Fortification of the flour with thiamine seemed to improve somewhat the appearance of hepatic cells around the central veins, and the increment of riboflavin was of further value. Among animals eating diet J a nearly normal hepatic cellular organization was maintained (fig. 3). Some peripheral necrosis developed but fat stains did not indicate degenerative changes. Fatty livers, or hepatic cirrhosis such as occurred among animals fed diets low in choline (Best, '41; Blumberg and McCollum, '41; Spellberg, Keeton and Ginsburg, '42) with high fat and low protein percentages, did not develop among our animals. When rats are fed synthetic diets, low in casein but not necessarily high in fats, hepatic injury is produced consistently (György, '42).
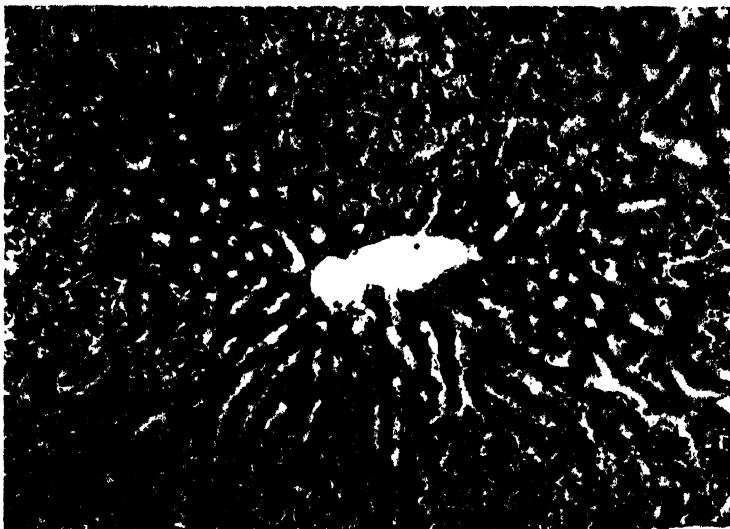
Fig. 2   Sections of liver of animal eating diet E (× 165). Peripheral necrosis of the hepatic cells had developed.
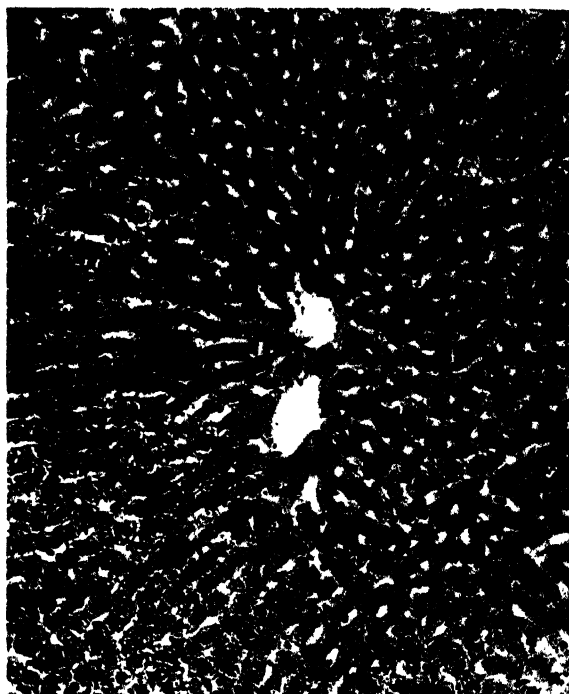
Fig. 3   Section of the liver of an animal eating diet J (× 165). A more nearly normal type of hepatic architecture was produced.

High casein ratios or the addition of methionine and choline exerted a considerable protective influence. The hepatic injuries observed in our rats fed these so-called human diets may be due in part to the protein-fat ratio (13.1 : 16.5). Although this percentage of fat (16.5) is not high, it is appreciably higher than that of our low thiamine human diet (10.8)
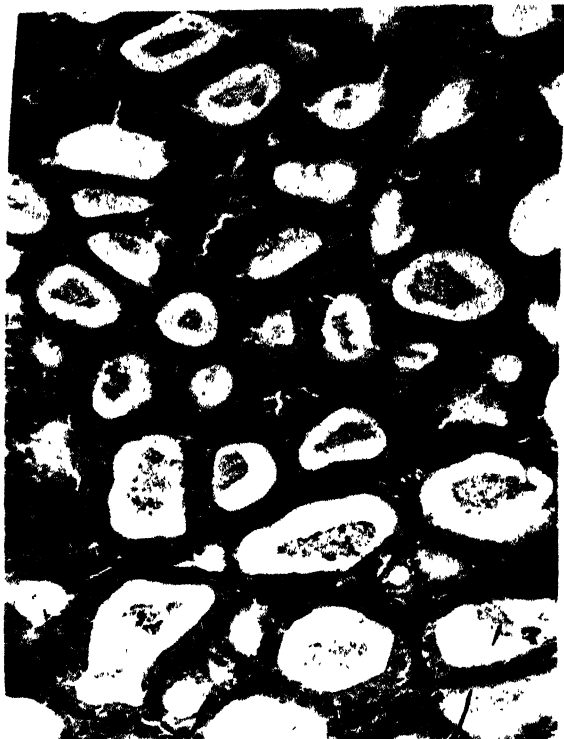
Fig. 4 Section of thyroid gland of animal eating diet E (× 200). Marked hyperplasia of the acini, with atrophic colloid masses, is shown.

and four times the fat percentage of our standard laboratory rat ration, in which the protein-fat ratio is 22:4.

The thyroid gland. Thyroid glands of all animals eating the experimental diets were hyperplastic (fig. 4). The follicles contained small amounts of colloid which was often contracted into amorphous masses and was stained blue with

Koneff's stain. Infrequently, the marginal colloid of such follicles had a reddish tinge. An acidophilic colloid was always observed in the thyroid glands of the control animals fed our standard ration. The follicular epithelium was more of the columnar type, often obliterating the lumina, in contrast to the normal flat or cuboidal type. The average height of the follicular cells in the thyroids of animals eating diet E was 8.2 microns; of those eating diet G, 12.7 microns; and of those eating diet J, 11.8 microns. The degree of hyperplasia
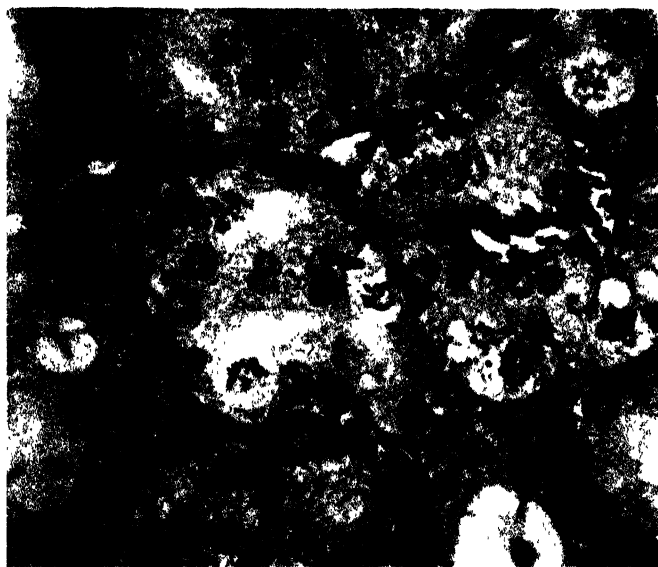
Fig. 5 Section of thyroid gland of animal eating diet G (× 450) Excessive hyperplasia was produced. Acinar lumina were obliterated and mitotic figures were common.

is apparent when it is recalled that the average height of cells in follicles of normal thyroids, in animals fed a standard ration was about 5.0 microns. On the contrary, even greater stimulation occurred in thyroids of animals eating diet G than occurred in those eating diet E. Cellular hypertrophy was sufficiently marked to occlude the lumina of the acini completely in the G group, and mitotic figures were abundant (fig. 5). The iodine content of the diets was not determined

but, since the salt used in the preparation of all diets was iodized, we have reason to believe that the iodine intake was probably adequate.

The pituitary gland. Differential counts of the cells in sections of the pituitary gland of some of the animals are shown in table 3. The data, while inconclusive, suggest that these deficient diets may have caused a reduction in the percentage of acidophilic cells and a corresponding increase in the percentage of chromophobes. The slight changes in the percentages of basophilic cells are not considered to have any special significance. Apparently the diets were incapable of maintaining the normal secretion of growth hormone. The addition of thiamine and riboflavin to the diets did not restore normal cell distribution in the pituitary.

TABLE 3

Percentages of cell types in the anterior lobe of pituitary.

DIET	ACIDOPHILS	BASOPHILS	CHROMOPHOBES
Laboratory ration	45.4	4.5	50.1
E	37.4	3.8	58.7
G	38.7	3.4	57.8
J	39.8	5.2	55.0

Vitamin assay of the tissues. Using the method of Hennessy ('41) for thiamine and that of Conner and Straub ('41) for riboflavin we determined the concentration of these vitamins in the liver, skeletal muscle, kidney and testes of five animals which ate diet E, and five which ate our standard ration for 6 months. These data are assembled in table 4. Concentrations of both thiamine and riboflavin in all tissues examined were significantly lower among the animals fed diet E than among the controls except for the riboflavin levels of the skeletal muscle and testes, and in these tissues the levels were statistically alike. Schultz, Light, Cracas and Atkin ('39) determined the thiamine concentration per gram of certain tissues of rats fed a synthetic diet for 28 days supplemented daily by (1) small amounts of thiamine (15 μg.) and (2) large amounts of

thiamine (215 μg.). Their group given the larger daily supplement of thiamine (215 μg.) had, after 28 days, 10 μg. of thiamine per gram of liver, 1.3 per gram of muscle, 6.7 per gram of kidney and 6.6 per gram of testes. These data correlate well with our own control data (table 4). In studies at the University of Texas ('41) concentrations of thiamine and riboflavin per gram of dry tissue were determined for the normal rat, microbiologic tests being used for the assays. When corrections are made for moist weights, our data, with few exceptions, compare well with those of these workers.

TABLE 4

Concentrations of thiamine and of riboflavin in tissues of conrol rats and of rats fed a diet low in thiamine and riboflavin.

ORGAN	THIAMINE CONCENTRATION μG./GM.		RIBOFLAVIN CONCENTRATION μG./GM.	
	Control	Diet E	Control	Diet E
Liver	9.7 (8.7 — 10.6) [1]	2.7 (2.2 — 3.1)	25.6 (23.2 — 28.8)	11.7 (10.0 — 14.6)
Skeletal muscle	1.6 (1.5 1.7)	0.44 (0.36 0.49)	1.0 (0.92 — 1.1)	1.2 (1.1 - 1.4)
Kidney	6.5 (5.9 — 7.7)	1.9 (1.6 — 2.1)	29.2 (27.9 — 32.5)	22.2 (18.3 — 24.2)
Testes	7.2 (6.6 — 8.1)	4.9 (3.9 5.6)	2.7 (2.4 — 3.2)	3.1 (2.7 - 3.6)

[1] Figures in parentheses indicate range of values.

SUMMARY AND CONCLUSIONS

This report covers some of the results of a study of feeding white rats a human diet, composed of foods commonly appearing on American tables, wherein the thiamine and riboflavin contents were low.

Five diets, differing from one another only in the flours from which the bread component was made, were prepared. Supplementations of patent white flour with thiamine, riboflavin and niacin, while improving the growth rate of the rats, were not adequate to secure weights attained by animals eating the diet that contained whole wheat flour.

The addition of thiamine to the flour increased the daily caloric intake from 26.8 to 40.0 and yet the average gain of weight was less than 1 gm. per day for the period when the intakes of food and water were tested. The low daily intake of riboflavin was inadequate for satisfactory growth.

The addition of both thiamine and riboflavin to the diet prevented the hypochromic anemia which developed among rats which ate either diet E or diet F. Essentially normal blood values were obtained by fortification of the flour with these two vitamins as well as by the diet which contained the whole wheat flour.

Peripheral necrosis associated with mild fatty degeneration occurred in the livers of all animals eating diet E. Cirrhosis did not develop. The addition of thiamine and riboflavin appeared to prevent extensive necrosis but the livers of rats eating the diet that contained whole wheat flour had a more nearly normal cellular organization than those of rats to whose diet thiamine and riboflavin were added.

The thyroid glands of all animals were hyperplastic. Mitotic figures were common in the acini of the thyroids of animals eating diet G. Extensive cellular activity was demonstrated even in animals eating diet J. These changes may be due, not to any lack of vitamins or to inadequacy of iodine, but perhaps to some dietary imbalance.

Changes from the normal in the percentage distribution of cells in the pituitaries were observed in these animals. These changes constituted relative increases of the percentages of chromophobes and corresponding decreases of the percentages of acidophils.

Chemical analyses of the concentrations of thiamine and riboflavin of the livers, skeletal muscles, kidneys and testes of animals eating diet E were made. These values showed excessively low concentrations of these vitamins in the tissues analyzed. They compared favorably with such data assembled elsewhere.

Fortification of the flour used in the bread component of the low thiamine-low riboflavin human diets with thiamine, ribo-

flavin and niacin in the amounts indicated proved inadequate to promote satisfactory growth or to prevent pathologic changes in the livers, thryroids and pituitary glands of white rats.

ACKNOWLEDGMENT

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THE ROLE OF BIOTIN AND "FOLIC ACID" IN THE NUTRITION OF THE RHESUS MONKEY [1]

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FOUR FIGURES

INTRODUCTION

It has been pointed out in a previous paper (Waisman et al., '43) that young monkeys (macaca mulatta) fail to grow and develop on a diet consisting of sucrose, purified casein, mineral salts, corn oil and cod liver oil together with adequate levels of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, para aminobenzoic acid (PABA) and i-inositol. On such a diet there is a gradual loss of weight followed by a syndrome of anorexia, leucopenia, lowered resistance to secondary infections, increasing cachexia and death. When liver extract, whole liver or a "solubilized liver" (commercial enzymically hydrolyzed) preparation was included with the "synthetic" diet, remission of all symptoms was noted and the animals showed striking weight gains. The complete absence of symptoms in those monkeys receiving the liver diets for 2 years is evidence that some factor (or factors) in the liver preparations was active in preventing the deficiency

syndrome in the animals. In this report evidence is presented that both biotin and the norite eluate factor (Hutchings et al., '41) or, as it is also known, "folic acid" (Mitchell et al., '41), are at least two factors required by monkeys in addition to the known crystalline and readily available vitamins.

EXPERIMENTAL

The diets fed throughout these studies together with the methods used for the care and handling of the animals have already been described (Waisman et al., '43). The weights of the animals together with daily observations of each animal were the guides used in determining when therapy was indicated. The animals which lost weight and failed to drink the vitamins or the particular concentrate were given the material by stomach tube until sufficient improvement resulted so that the supplement was consumed voluntarily.

The biotin used in these experiments was a commercial concentrate [2] containing 20 µg. per milliliter. The "folic acid" concentrate was the norite eluate of liver prepared according to the method of Hutchings et al., ('41) through the first norite absorption and elution with the ammonia-alcohol mixture. Each preparation of the concentrate was microbiologically assayed [3] by the Streptococcus lactis method (Mitchell and Snell, '41) and the activity expressed as percentage potency of a standard solubilized liver. The majority of the preparations ranged in activity from 35 to 65%, but an occasional preparation was only 20% as active as solubilized liver. The various liver products and liver concentrates were also microbiologically assayed both for their biotin [4] and "folic acid" content.

The growth data were obtained with young monkeys who were initially depleted of their stores of the various food factors by giving them the basal synthetic diet with the known crystalline vitamins until the first constant drop in weight was

[2] S.M.A. Co., Chagrin Falls, Ohio, no. 200.
[3] By Mr. T. D. Luckey.
[4] By Mr. D. Miller.

observed. They were then given the "folic acid" concentrate daily in the solution containing the vitamins for periods of a week to 2 months or more until a definite conclusion could be reached as to its effectiveness as a growth-promoting concentrate. Control animals received the material from the beginning. Red and white cell and hemoglobin determinations were made at convenient times, commonly every 2 weeks, upon blood samples drawn from the marginal veins of the ears. The white cell counts were made at the same time daily during the deficiency and throughout the period of "folic acid" therapy.

Biotin studies

It has been shown (chart 2, Waisman et al., '43) that the addition of 20 µg. biotin per day to the synthetic ration did not prevent the decline in weight nor improve the condition of the animal in any way. While no growth effects can be attributed to biotin under the experimental conditions employed, the results appear convincing that this vitamin is concerned in some way in the maintenance of normal fur.

A total of eleven monkeys were used in experiments involving biotin. Some monkeys received 20 µg. from the beginning of the experiment, several received the biotin only after nutritional failure became apparent, and others received the biotin together with various liver fractions. The five animals which received 20 µg. biotin per day together with the nine crystalline vitamins and the synthetic diet from the start showed the same syndrome of nutritional failure as though the animals were fed the diet and the nine vitamins without biotin. These animals declined within the 30–85 days noted previously; similarly, several other animals which received the biotin when the weight curve showed a plateau showed no delay in the onset of nutritional failure. It is apparent that biotin is not the factor which maintains monkeys fed the synthetic diet, nor does it appear to be concerned in delaying the symptoms previously described when no other fractions were fed.

In our previous paper the experimental procedures were described under which we kept twelve monkeys in excellent nutritive condition for nearly 2 years on synthetic diets containing 3% liver extract. In these monkeys the fur was normal in texture, color, quantity, and brilliance. In contrast to this, the monkeys maintained on 3% solubilized liver gradually lose their fur color, then lose areas of fur leaving a denuded portion in which there may be occasional dermatitis, and finally
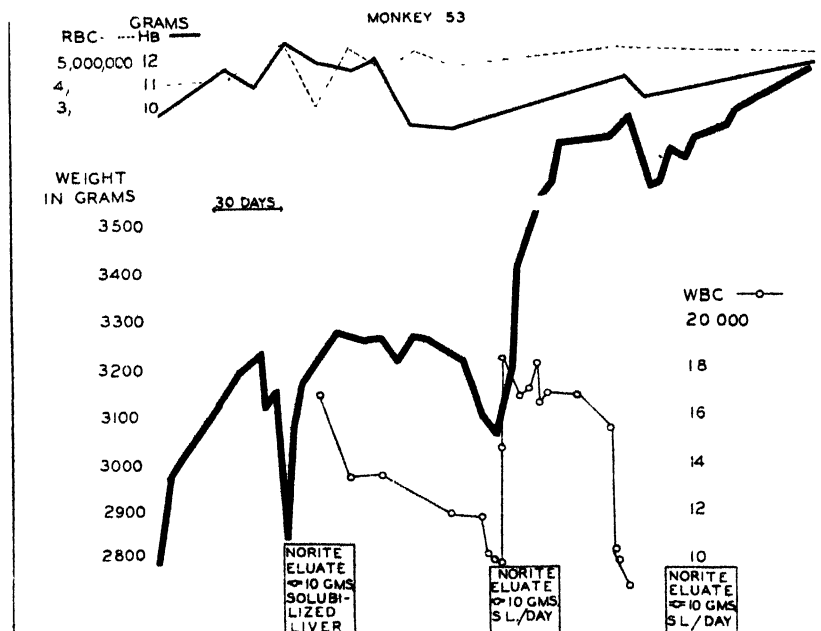
Fig. 1   Typical growth responses and white blood cell increase following the administration of a norite eluate of solubilized liver (''folic acid'' concentrate).

the entire body surface will be largely denuded and the monkey will have a blanched appearance with the skin markings clearly visible through the short guard hairs that remain. These gradual changes are shown in figures 2, 3, and 4. The time of onset of this denudation and off color appearance of the fur is variable, apparently dependent upon the quality of the diet fed prior to the experimental period in question. The six monkeys fed 3% solubilized liver became denuded in from 2 to 7 months.

The administration of 20 µg. of biotin per day to the denuded animals resulted in a gradual return of the fur; within 30 days definite areas were filled with long hairs and after 60 days much of the color returned. In several monkeys after 4 months of biotin therapy the fur was completely restored but the

Figure 2

Various stages in the loss of fur in monkeys receiving the solubilized liver diets are illustrated in this figure, as well as figs. 3 and 4.

original color was not attained. It is not possible to say at this time whether another factor is involved but it should be remembered that in those monkeys kept on the basal diet plus 3% liver extract, the fur retained its normal golden brown color; it should also be noted that together with the loss of hair and a definite dry dermatitis, a "rusty" appearance of the

skin appeared either prior to the alopecia or concurrently with it. This "rusty" condition is very likely due to the porphyrin-type compound described as the material in the "bloody tears" of pantothenic acid deficient rats (McElroy et al., '41). In those monkeys fed biotin, this "rusty" condi-
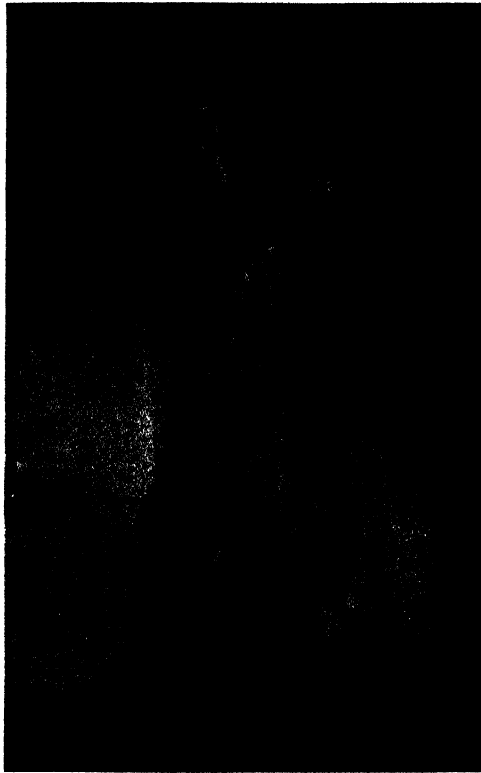
Figure 3

tion completely disappeared shortly before the hair began to grow back. The alopecia develops either during good growth on diets containing 3% of solubilized liver or during poor growth and even weight loss on diets containing 1% of the same. To illustrate, a monkey getting 1% solubilized liver showed discolored fur and denudation long before the loss in weight which occurred because of the inadequacy of "folic

acid" (to be described later) on the 1% level of solubilized
liver. Biotin feeding was started 2 months after the monkey
was placed on the 1% solubilized liver diet and the fur began
to grow in slowly although the monkey showed a gradual loss
in weight. At the time the diet was changed to 3% solubilized

Figure 4

liver the fur was nearly normal in amount although still a
little off color in spots.

An interesting observation was made on two animals which
received 1% solubilized liver diet but no added pantothenic
acid. After a short period of growth, the animals gradually
lost weight and small areas of baldness were visible on the
sixty-ninth day when a "folic acid" concentrate equivalent to
10 gm. of solubilized liver was fed to prevent further weight

loss. The first animal began to grow again but became increasingly denuded until, after 60 days more, it was completely lacking in hair. Continuing the "folic acid" supplement, growth was nearly normal and, 95 days after the greatest hair loss was first observed, a slow growth of hair had begun. In another 30 days the hair growth was quite profuse with only a few bald patches remaining. The color was abnormal and the texture faulty. It should be remembered that at no time did this monkey receive biotin. The other monkey paired on this experiment also lost its fur but in a slightly longer period of 150 days. This animal was then fed 3% solubilized liver, the pantothenic acid still being omitted from the daily supplement, and although the weight of the animal was increased over that gained on 1% solubilized liver, the fur continued to be sparse and only a portion of the shoulder was covered with fur of a lighter color. The "folic acid" supplied by the 3% solubilized liver appears then insufficient to give the fur growth that the "folic acid" concentrate supported. This assumption is borne out by the fact that animals getting 3% solubilized liver become denuded while those animals given "folic acid" concentrate equivalent to 10% solubilized liver did not show this denudation during the period of our experiments. It is noteworthy that one of our monkeys fed the 3% solubilized liver diet but getting no pantothenic acid for more than 15 months has shown no change in fur color or texture.

"Folic acid" results

An appraisal of our data early in 1942 after 8 months of feeding a variety of diets led us to believe that there was a difference in the growth of monkeys fed the synthetic diet and all nine crystalline vitamins and those fed varying amounts of liver preparations, grass extracts and a ration containing heated corn and wheat middlings (Waisman et al., '42). Liver extract was chosen as the starting product for fractionating work in an attempt to identify the active factor or factors. It was found that the factor was stable to alkali, not extracted by prolonged continuous ether extraction in acid solution, ad-

sorbed by norite and eluted with alcohol and mild alkali. With these data in mind and with the aid of microbiological assays of our diets and a knowledge of liver preparations and fractions which had accumulated in this laboratory, we were led to the conclusion that the factors in liver and grass were probably identical with the factor called "norite eluate factor" by the Wisconsin group (Hutchings et al., '41) and therefore with the factor named "folic acid" by the Texas group (Mitchell et al., '41). The feeding of a concentrate of the norite eluate to monkeys which showed the weight loss characteristic symptoms (leucopenia, anorexia, etc.) showed first an increased appetite which reflected itself in definite weight gains and, second, a sharp increase in white blood cells to the normal level for the monkey.

At this writing more than twenty-five trials with at least fifteen monkeys fed the "folic acid" concentrate have convinced us that this preparation contains a factor or factors essential for normal growth in this animal. In most of our trials the equivalent of 10 gm. of solubilized liver was fed, but we have obtained equal responses in several monkeys with an equivalent of 5 gm. per day. The response to the "folic acid" concentrate was considered reliable since in a sufficient number of animals the basal diet was first fed until nutritional failure resulted and this was followed by the feeding of either the 3% liver extract or 3% of the solubilized liver diets. After an adequate response was obtained the animal was returned to the basal synthetic diet until nutritional failure again resulted and then the "folic acid" concentrate was given. This procedure was reversed in several cases, the "folic acid" being given first and followed by either the 3% liver extract or solubilized liver.

That 1% solubilized liver supplies inadequate "folic acid" for growth and maintenance is shown by the nutritional failure and by the response which can be obtained in those animals when they are fed a "folic acid" concentrate equivalent to 5% liver. The feeding of the "folic acid" concentrate to an animal already cachectic and moribund delayed the time of death for

a week or 10 days beyond that period found previously. Although life was prolonged, the hemorrhagic condition of the digestive tract due to the dysentery may have accounted for the inability of the animal to absorb fully and therefore respond to the supplement.

Monkeys fed from the beginning with "folic acid" concentrate equivalent to 5 or 10 gm. of solubilized liver together with the crystalline vitamins and the synthetic diet, have shown good growth for more than 130 days and are still on experiment. Other monkeys have been given the "folic acid" concentrate with 20 µg. of biotin from the start and have been maintained now for more than 266 days. This type of experiment is indicative of the adequacy of the supplements to the synthetic diet; also of the adequacy of the "folic acid" concentrate to supply the essential factor or factors for maintaining normal growth and well-being beyond the time when the animal would ordinarily be expected to fail if it were given the synthetic diet alone. In order to gain additional information on the specific factors furnished by the "folic acid" concentrate, it will be necessary to attempt further fractionation and isolation procedures.

Along with the response in growth, the second effect of the "folic acid" supplement has been to restore the normal blood picture in the deficient monkey showing leucopenia. The daily white cell count was followed during most of the "folic acid" trials in the monkeys, together with the counts in those monkeys which served as controls (those getting 3% solubilized liver described in this and the preceding paper). As will be seen in figure 1, typical responses of white blood cell formation have been obtained following "folic acid" (norite eluate) therapy. The white cell increase follows very closely the feeding of the "folic acid" containing supplements. Each monkey must necessarily serve as its own control since the normal white cell count varies from animal to animal, as found by Shukers et al. ('38) and confirmed in this laboratory. The removal of the "folic acid" supplement resulted in the gradual loss in weight, but it appeared that the white cell count

dropped before extreme deficiency symptoms developed. No differential counts were made in this study since we were interested in the total response which could be elicited by the "folic acid" concentrate. Wilson et al. ('42) found that a commercial "folic acid" concentrate was effective in establishing a normal white cell equilibrium in two monkeys.

DISCUSSION

The growth response which has been noted in deficient monkeys when a "folic acid" concentrate was fed is adequate demonstration that this preparation contains the factor or factors found in the three liver products previously described. The curative trials in which the "folic acid" was fed in amounts equivalent to 10 gm. solubilized liver gave as effective responses as the 3% original solubilized liver. This appears valid since the norite eluates contained on the average approximately 40–50% of the original activity, and the monkeys consumed approximately 200 gm. of the 3% liver diet. The "folic acid" supplement aided in overcoming the dysentery fully as well as the original liver products in the deficient animals. Since the white cell count increased consistently upon the addition of the "folic acid" to all deficient monkeys, it is convincing evidence that this concentrate contains the factor responsible for the remission of the leucopenia in monkeys fed liver.

Experiments are still in progress in which the "folic acid" is the only supplement other than the nine crystalline vitamins, fed from the beginning to monkeys; however, these experiments have gone far enough to support the conclusions drawn. Although these animals were not fed biotin, the data so far obtained indicate that the monkeys are able to grow on the synthetic diet alone and be maintained in apparent good health for a period longer than the maximum time at which nutritional failure can be expected.

Monkeys fed 1% liver extract are able to grow and survive in contrast to those which fail on diets containing 1% solubilized liver. This observation may be important since it not

only points out the differences in the two liver products but also furnishes some evidence that liver extract supplies the necessary nutrients for the favorable intestinal bacteria. This notion is supported by the finding that monkeys, failing on the 1% solubilized liver diet and then fed the "folic acid" concentrate, are able to recover and resume growth, thus indicating that 1% solubilized liver does not furnish sufficient quantities of the factor. At the 3% level, adequate amounts of the factor(s) are supplied so that there are no apparent discrepancies in the growth between the animals getting 3% solubilized liver and 3% liver extract.

There are at present no means of determining with any finality that the "folic acid" concentrate as prepared contains only the one physiologically active compound named "folic acid". It is difficult at this time to refer the growth response, the remission of the leucopenia, and perhaps also the prevention of the dysentery to only one substance in the liver concentrate, but until pure compounds are isolated and shown to be physiologically active, it appears unwise to attribute each observation to a separate substance.

The question of fur loss calls attention to several noteworthy points. With but one exception in nearly twenty animals receiving 3% liver extract, all the monkeys showed normal fur texture and color. Liver extract fed as low as 1% was able to maintain normal fur and skin structure. With 1% solubilized liver however, the animals became completely blanched and denuded. The gradual loss of fur and final denudation were independent of body growth in monkeys as shown by the continued growth of those animals getting 3% solubilized liver. The gradual return of some fur was noted after 3 months in one animal given the 1% solubilized liver diet and "folic acid" concentrate, although no biotin was fed. Another monkey paired on this experiment also lost all its fur but had not received any "folic acid" concentrate and at this writing remains nearly completely denuded. If the role of biotin is a specific one, then this study, showing the necessity of supplying this factor to monkeys for normal skin and fur structure,

is one of the first experiments in which raw egg white is not used to demonstrate that experimental animals require biotin. In this laboratory, Cooperman, Waisman and Elvehjem ('43) have presented evidence that hamsters require biotin when fed a diet containing no raw egg white. Sydenstricker et al. ('42) and Oppel ('42) have reported on biotin studies in man.

Some quantitative relationships of the factors involved are brought into focus when one considers the denudation which occurs even in those animals receiving 3% solubilized liver which ostensibly furnishes adequate "folic acid" for growth while the "folic acid" equivalent to 10% solubilized liver stimulates fur growth in monkeys getting the 1% solubilized liver diet. The solubilized liver and liver extract contain approximately the same quantity of "folic acid" but the biotin in liver extract is about five times that which occurs in solubilized liver. Solubilized liver then contains not only insufficient biotin but also an inadequate quantity of the factor in liver extract which may be necessary for the formation of biotin by intestinal bacteria. The exact level of biotin necessary for fur replacement is not known since up to this time we have used only the 20 µg. per day supplement. The possible role of the interrelationship of the various vitamins in influencing the growth of intestinal bacteria, which in turn synthesize known and unknown factors then available to the animal, is shown by the finding of a normal skin and fur in a monkey getting no pantothenic acid other than the small amount supplied in the 3% solubilized liver diet. The ability of biotin to cause this regrowth of fur is clearly evident, but the role of other factor(s) in either stimulating synthesis of biotin or in supplying other substances per se is not fully established.

SUMMARY

1. The nutritional failure which results in monkeys fed a "synthetic" diet plus eight crystalline members of the B group of vitamins and ascorbic acid can be readily cured or prevented by the feeding of a norite eluate fraction of liver, also called "folic acid".

2. Normal growth is observed in monkeys given only the "folic acid" concentrate in addition to the basal diet and the nine crystalline vitamins.

3. The leucopenia in the deficient monkeys is quickly alleviated by the administration of the "folic acid" preparation.

4. Monkeys kept on 1 or 3% solubilized liver for long periods show loss of hair and nearly complete denudation together with a slight dermatitis and "porphyrin-like" secretion on the skin. These changes are alleviated by the administration of biotin.

5. Biotin has no growth-stimulating properties under the experimental conditions described.

ACKNOWLEDGMENT

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LOSSES OF VITAMINS IN LARGE-SCALE COOKERY [1]

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Very little work has been done on vitamin losses in large quantity cookery. The estimated loss of thiamine and ascorbic acid in the restaurant cooking and holding of vegetables is usually given as about 75% (Nagel and Harris, '43).

The present study was conducted at one of the cafeterias feeding industrial workers at the Boooklyn Navy Yard. The purpose of this study was to give a rough estimate of the losses in vitamins in the actual field of large-scale cookery. It is in no sense a carefully controlled study to locate the steps in the cookery that cause these losses.

Most of the vegetables used in this cafeteria were frozen — usually green lima beans, green string beans, spinach and green peas. Raw vegetables used were potatoes, carrots and turnips; canned vegetables were mostly corn and sauerkraut. Canned beets and carrots were used occasionally.

Table 1 gives the vitamin values per 100 gm. of the material as purchased, during the months of November and December,

1942. The values on the vegetables compare very favorably
with those already reported, with the exception of cabbage.
Frozen lima beans gave variable results. However, the beans
were not consistent in either color or size, hence the wide
variation in values. For example, the ascorbic acid varied
from 3.9 to 15.4 mg. per 100 gm.; the vitamin A from 33 to 316
I.U. per 100 gm.

TABLE 1

*Vitamin values for raw and frozen vegetables used at the
Brooklyn Navy Yard, per 100 gm.*

VEGETABLE	CAROTENE	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
	Expressed as I.U. of vitamin A	*μg.*	*μg.*	*μg.*	*mg.*
Peas, frozen	500–566	332, 368 303	120	2.36	11.1, 11.4
Lima beans, frozen	33, 316	119, 79, 110, 85	64, 80 100	0.85, 0.82, 1.34	13.1, 4.5 3.9, 15.4
Green beans, frozen	83, 550	56, 73, 86, 96	100, 100 130	0.26, 0.42, 0.52	4.0, 9.8, 11.5
Spinach, frozen	9280	90, 101, 114	190	0.49	20.8, 34.9
Carrots, raw, old	9283	82	50	0.48	5.3
Potatoes, raw	neg.	53, 71, 82 82, 90, 62	30, 30	0.32, 0.54	8.7, 15.0
Cabbage, raw	neg.	71	30	0.27	4.3

The carotene, niacin, riboflavin and ascorbic acid values
were determined at the U. S. Plant, Soil and Nutrition Labora-
tory at Cornell University. Carotene was determined by the
chromatographic method (Moore and Ely, '41; Strain, '38;
Curtis, '42), niacin by the microbiological method of Snell
and Wright ('41), riboflavin by the microbiological method
of Snell and Strong ('39), and ascorbic acid by the chemical
method with a modification described by Morell ('41); the
aliquot, however, was titrated to an end point with standard-

ized dye. Thiamine was determined by two different methods. Duplicate samples were sent to Fordham and Cornell Universities. Dr. L. R. Cerecedo of Fordham used the thiochrome method of Hennessy and Cerecedo ('42); the laboratory at Cornell used the fungus assay method first described by Schopfer and Jung ('37) and modified by Hamner ('43).

There was exceptional agreement on the thiamine values for vegetables from the two laboratories. This comparison is given in table 3, for the food as served.

Samples of vegetables were collected as follows: (1) frozen product before defrosting, or a sample of the raw product; (2) cooked product, immediately after cooking; (3) cooking water, immediately after vegetables cooked; and (4) steam table sample at 12 o'clock noon, when the serving ends.

In table 2 is recorded the total loss of vitamins from the vegetables prepared. The losses of vitamins in the vegetables during the cooking and holding processes are variable. Excluding the potato, the thiamine losses were 16 to 64%, with an average of 37; niacin losses 2 to 61%, average 27; riboflavin losses 22 to 45%, average 36; ascorbic acid losses 27 to 90%, average 65. While this research was being conducted, a trained home economist was working in the kitchen on the problem of the best methods to employ in cooking vegetables in mass feeding. Therefore, the amount of water used in cooking a given amount of vegetables, the cooking time, and the holding time after cooking had not been completely standardized.

It was not possible to estimate the exact losses in the different steps of preparation, but determinations for ascorbic acid indicate that of the total per cent lost, 3 to 65%, with an average of 21, was lost in the cooking water; 24 to 77, average 56%, destroyed in the cooking process; 0 to 67, average 22% destroyed on standing.

Table 3 was prepared to show what and how much of the various vitamins the customer actually got from a serving of the different vegetables as prepared in the cafeteria kitchen.

TABLE 2

Vitamin losses in cafeteria-prepared vegetables.

VEGETABLE	COOKING METHOD	AMOUNT Prepared	AMOUNT Water used	COOKING TIME	HOLDING TIME [1]	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
		lbs.	gal.		min.	%	%	%	%
Peas, frozen	Boiled	10	½	27	38	25	38	41	68
Peas	Boiled	20	(?)	20	130	50	..	..	65
Lima beans, frozen	Boiled	60	9½	50	165	63	..	..	72
Lima beans, frozen	Boiled	20	1¼	25	130	37	34	22	44
Lima beans, frozen	Boiled	40	4½	40	50	43	35	45	27
Lima beans, frozen	Boiled	20	1½	33	50	35	33	35	77
Green beans, frozen	Boiled	20	2¼	45	115	55	..	..	..
Green beans, frozen	Boiled	40	4	25	120	43	4	30	..
Green beans, frozen	Boiled	20	1½	27	58	15	36	34	66
Green beans, frozen	Boiled	20	1½	29	68	33	15	34	74
Spinach, frozen	Boiled [2]	40	3	45	90	64	..	..	80
Spinach, frozen	Boiled	20	¼	23	17	22	22	..	65
Potatoes, raw	Steamed	85	..	60	65	2	10	10	90
Potatoes [3]	Steamed	300	..	40	30	7	2	2	51
Carrots, raw	Boiled	100	4	45	..	52	61	45	23

[1] Time between completion of cooking and the serving time (11: 30 A.M.)

[2] Spinach defrosted in several gallons cold water. Water drained off and spinach cooked, in fresh boiling water. There was 49% loss of thiamine and 52% loss of ascorbic acid by defrosting, before cooking.

[3] Potatoes (both samples) peeled day before used, covered with cold water, put in refrigerator overnight. Water drained before steaming — hence the large losses of ascorbic acid. Thiamine in several samples of potatoes tended to increase in value. Milk was added when potatoes were mashed.

TABLE 3

Vitamin content of a serving of cafeteria prepared vegetables.

VEGETABLE	SIZE PORTION	VITAMIN A	THIAMINE	RIBO-FLAVIN	NIACIN	ASCORBIC ACID
	gm.	I.U.	µg.	µg.	mg.	mg.
Peas, frozen	85	496	220	80	1.45	3.06
Peas, frozen	85	567	150	..	...	3.40
Lima, beans, frozen	85	240	40	..	...	3.23
Lima beans, frozen	85	...	40	40	0.48	1.79
Lima beans, frozen	85	14	50	40	0.45	2.47
Lima beans, frozen	85	240	50	40	0.77	2.96
Green beans, frozen	85	42	50	80	0.21	3.74
Green beans, frozen	85	496	40	60	0.23	2.89
Green beans, frozen	85	467	60	60	0.37	2.55
Spinach, frozen	85	8216	30	..	...	3.49 [1]
Spinach, frozen	85	7450	60	..	0.32	14.53
Potatoes	112	0	80	60	0.32	1.68
Potatoes	112	0	100	80	0.40	3.80
Potatoes	112	0	100	40	0.59	4.80
Potatoes	112	0	100	80	0.40	4.70
Cole slaw	85	neg.	50	30	0.33	3.15
Carrots	85	...	30	20	0.13	4.08
Carrots	85	9605	30	30	0.16	3.49
Turnips	85	28	53	30	0.33	7.82

[1] See footnote, table 2, for method of defrosting.

DISCUSSION

Some problems in industrial feeding

Although menu planning and food selection are important, other factors which help to improve the nutritive value of the meals served are kitchen management and improved methods of food preparation. The observations mentioned here were made in only one cafeteria. This particular establishment prepared food for an office worker's dining room serving approximately 1400 people, as well as their own dining room serving 1000 to 1400 laborers.

In the present case operations were improved as much as possible while the study was in progress, and after it was completed, in order that the vitamin values might be conserved as much as possible. This involved decreasing the holding time between preparation and serving. Even with inexperi-

enced labor this was possible since meals were served according to a definite schedule.

In general, schemes that help speed service are: (1) posting menus in large print in strategic places so that the customer can make his own choice before he gets to the counter; (2) decreasing daily choice as much as possible; adequate choice can be extended over a period of time in the menus; (3) making the main dish choices as distinct from each other in appearance, flavor and cost as possible. When they are too much alike it causes confusion and indecision.

Decreasing choice aids in kitchen management. The preparation of fewer items simplifies the kitchen schedule, improves the work of untrained help and increases the efficiency in the use of limited equipment.

Decreased waste of food resources results from tested recipes which insure certain nutritive values per serving, such as protein and calories, and, as far as possible, minerals and vitamins.

SUMMARY

Preliminary studies were made in a large industrial cafeteria to determine the losses in vitamins in vegetables under actual operating conditions. The losses, in per cent, ranged as follows: thiamine 16 to 64; niacin 2 to 61; riboflavin 22 to 45; ascorbic acid 27 to 90. Changes were then made in the vegetable cookery by decreasing radically the time of cooking large quantities. The time of holding vegetables between cooking and service was also cut to a minimum. Texture, flavor and appearance were improved.

A comparison of the thiochrome and fungus assay methods for the determination of thiamine gave excellent results for analyses in vegetables.

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ADEQUACY OF THE INDUSTRIAL LUNCH AND THE USE OF BREWER'S YEAST AS A SUPPLEMENT

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The purpose of the present study was to determine the adequacy of the vitamins in the noon meal consumed by a group of workers in the Brooklyn Navy Yard. After this was determined, brewer's yeast was introduced into certain cooked products to increase the water soluble vitamins in this noon meal.

The problems of feeding in a large plant such as the Navy Yard are numerous. The distance between the various cafeterias is great; the time for eating is short; the number of men is large; there are many problems in cookery. All contribute to the difficulty of supplying a third of a day's requirements in vitamins. One method of helping to overcome this difficulty is to increase the vitamins in the food by the addition of some natural product such as yeast.

Until relatively recent times man has been accustomed to consume yeast in various forms such as that found in beer. The beer now produced is very clear and quite free from yeast. Other uses are worthy of consideration. This problem has received our attention.

In preparation for the use of yeast in foodstuffs, several months were devoted to testing yeast in various forms and recipes. Yeast makes pleasing combinations with syrups or peanut butter. It can also be used in baked products such as breads, cookies and doughnuts. Considerable amounts of

yeast can be incorporated into meat loaves, stews, cheese dishes, baked beans and meat balls. Yeast cannot be used in corn bread, biscuits or in other mildly flavored recipes.

Yeast has been converted into both tablets and pellets. The most satisfactory combination for these consists of equal parts of dry skim milk and dry brewer's yeast. The cost of manufacturing pellets is only a small fraction of that for tablets.

To a limited extent dry yeast has been employed in human foodstuffs for many years, especially since its value has been appreciated in treating pellagra. Carr ('41) has published recipes for the use of yeast in home cookery. Johnson and others ('42) found yeast useful in providing a source of thiamine in their studies of exercise. For many years yeast has been employed in various studies at Cornell (Savage and McCay, '42). In the course of this work its merit has been appreciated not only because it is an inexpensive natural source of recognized vitamins but also because many experiments indicate that it contains essential factors that have never been isolated and recognized.

<center>EXPERIMENTAL</center>

The studies reported here were made in the kitchens and cafeteria of the Brooklyn Navy Yard. Complete meals as well as the various items offered in the menu were selected in such a manner as to get representative samples. After weighing, these were put through a Waring Blendor. Two aliquots were then packed in dry ice. One was shipped to Fordham University and the other to Cornell. Thiamine was determined at both Fordham and Cornell; niacin and riboflavin were determined at Cornell, by the methods described elsewhere (Heller et al., '43).

Two types of meals were studied, namely cafeteria meals and a lunch [1] prepared and then packaged in individual paper containers, kept warm in an oven until lunch time and then served on paper trays, from outdoor stands. They usually consist of 7 to 10 ounces of soup, a main dish (such as a stew,

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[1] Mealpak.

beans and frankfurters, macaroni and cheese, etc.), two slices of white enriched bread, a pudding or jello dessert, occasionally a small vegetable salad, and a choice of either milk or coffee.

The lunches are employed to help speed the distribution of food and to overcome the disadvantage of the distance between groups of workers.

The cafeteria meals usually consist of a roast meat, meat-vegetable dish, or meat alternate such as macaroni creole, etc., mashed potatoes, one other vegetable (such as green lima beans, green peas, green beans, carrots, spinach or turnips), two slices of rye, whole wheat or enriched white bread, one pat of butter, choice of dessert (usually pie), and choice of coffee, tea or milk.

All the men eating in the cafeteria do not select such a hearty meal as described above. A large number of them do, but many bring their sandwiches from home and supplement them with coffee, and dessert from the cafeterias or stands. Many of the naval personnel eating in the cafeteria select milk as a beverage. About one-fourth to one-third of the workers select milk.

The results of the thiamine determinations are shown in table 1. The fungus assay method applied for thiamine analyses in whole meals gives higher values than the thiochrome technique. This contrasts with the excellent results for vegetables by the two methods, reported by Heller and co-workers ('43). The error due to meat must be much larger than that indicated for the whole meals, since the accuracy on vegetables would reduce the discrepancy in the meal. In the following discussions, the fungus assay values have been used.

The results of all the vitamin determinations are summarized in table 2.

DISCUSSION

The noonday meal fails to furnish a third of the day's vitamin needs if the accepted standards are correct. The morning meal cannot be expected to furnish a third unless eating habits are much improved. Breakfast can be improved by using better

bread for toast, more citrus fruit, and doughnuts made with liberal additions of such products as soy bean flour and brewer's yeast.

After these initial studies indicating the level of vitamins ingested in the noonday meal, dried brewer's yeast was added to certain of the cooked products to see if it could be incorpo-

TABLE 1

Thiamine content of lunches[1] and cafeteria meals. Comparison of values from thiochrome and fungus assay methods.

CAFE-TERIA MEAL NO.	FUNGUS ASSAY (CORNELL)	THIOCHROME (FORDHAM)	LUNCH NO.	FUNGUS ASSAY (CORNELL)	THIOCHROME (FORDHAM)
	μg./gm. fresh wt.	*μg./gm. fresh wt.*		*μg./gm. fresh wt.*	*μg./gm. fresh wt.*
I	...	1.55	I	0.22	...
II	0.79	0.46	II	0.28	...
IV	0.66	0.47	III	0.58	...
V	0.68	0.45	IV	0.19	...
VI	0.94	0.62	V	0.34	...
VII	0.59	0.44	VI	0.46	0.31
VIII	...	1.26	VII	0.78	0.45
X	1.46	1.19	VIII	0.66	...
XI	0.61	0.49	IX	0.57	...
XII	0.73	0.54	X	0.84	0.52
XII	0.74	0.58	XI	0.90	0.62
XIII	...	2.89	XV	1.46	0.97
			XVI	0.41	0.27
			XVII	0.48	0.35
			XVIII	0.83	0.66
			XIX	0.71	0.53

[1] Mealpaks.

TABLE 2

Contribution of the noon luncheon to the day's needs.

TYPE OF MEAL	NO OF CASES	THIAMINE		RIBOFLAVIN		NIACIN	
		Mean (range)	Fraction of daily need [1]	Mean (range)	Fraction of daily need [1]	Mean (range)	Fraction of daily need [1]
		mg.	*%*	*mg.*	*%*	*mg.*	*%*
Lunch	16	0.54 (0.17–1.20)	23	0.59 (0.30–1.65)	18	3.37 (1.20–5.99)	15
Cafeteria	9	0.62 (0.39–1.06)	27	0.57 (0.30–1.65)	17	6.05 (2.49–9.65)	26

[1] Assumed dietary allowance for the working man: 2.3 mg. thiamine; 3.3 mg. riboflavin; 23.0 mg. niacin.

rated at a reasonable level without injury to the flavor and appearance of the food. Considerable initial effort was given to standardizing the recipes.[2] Yeast was added to the following: stuffed green peppers, meat loaf, meat balls, pork goulash, lima bean creole, veal or lamb curry, salmon salad sandwich spread, cheese sandwich spread and bean-frankfurter spread. It was also added to the beef goulash as well as to the beef-kidney stew by the chef.

TABLE 3

Comparison of thiamine content of main dishes with and without dry brewer's yeast, by thiochrome and fungus assay methods.

FOOD	SIZE OF PORTION	FUNGUS ASSAY (FRESH WT.)	THIOCHROME METHOD (FRESH WT.)
	gm.	*µg./gm.*	*µg./gm.*
Pork goulash	196	2.6	3.9
Pork goulash + yeast		14.7	10.7
Curry of veal	196	0.35	0.35
Curry of veal + yeast		7.5	7.9
Meat loaf	84	1.1	1.1
Meat loaf +yeast			19.8
Beef and kidney stew	196	0.81	0.48
Beef and kidney stew + yeast			4.4
Beef goulash	196	0.72	0.36
Beef goulash + yeast		3.73	3.15
Lima bean creole	196	1.59	1.09
Lima bean creole + yeast			4.23
Stuffed green peppers	112	1.2	. . .
Stuffed green peppers + yeast		14.3	15.09

The yeast added was a product rich in thiamine,[3] containing 723 µg. of the vitamins per gram. The increase in the thiamine obtained by adding brewer's yeast to some main dishes is shown in table 3. This table also gives the average helping of the different products.

Ordinary brewer's yeast contains the following vitamins per gram: thiamine, 150 µg.; riboflavin 50 µg.; and niacin 600

[2] Standardized recipes were developed by Marcia Wolf, Nutritionist for the Canteen Food Service.
[3] Supplied by the Yeast Products Corporation.

µg. With the very conservative additions of yeast that were used at the level of these initial attempts, the average increase in thiamine if ordinary yeast were used would be 0.55 mg. (range 0.20 to 0.90 mg.); corresponding figures for riboflavin and niacin would be 0.18 (0.07 to 0.30 mg.) and 2.18 (0.80 to 3.60 mg.) respectively.

Dry brewer's yeast could have been used at a higher level, especially if yeast with a mild flavor had been employed. The values given here probably represent a low starting level for large scale cookery.

Attempts were made to determine vitamin C and carotene contents of the composite samples of mixed foods from these meals. In both cases so many difficulties were encountered that the results are regarded as valueless. Carotene values in many cases tended to be higher in the cooked meal than in similar ingredients before cooking. In the case of vitamin C, also, the values for the meal were too high from calculations based upon the individual items in a given meal.

SUMMARY

The meals served workers in one cafeteria in the Brooklyn Navy Yard have been sampled for the determination of riboflavin, niacin and thiamine. This noon meal usually furnishes at most only one-fourth of the day's requirements for these vitamins. Dry brewer's yeast has provided a satisfactory method of supplementing the supply of these vitamins. This yeast was usually incorporated in the meat dishes at conservative levels, so that the taste was not detected. Since yeast is rich in protein of good quality it also affords a good source of this dietary factor.

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FACTORS INFLUENCING THE BIOASSAY
OF VITAMIN E [1]

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INTRODUCTION

The establishment of a standard method of vitamin E assay has been in progress since 1927, when Evans and Burr made observations of the fetal resorptions in female rats deprived of vitamin E. Bacharach ('38) defined the mean fertility dose as that dose showing a response of a live litter in 50% of the implanted animals. Bacharach and Allchorne ('38) define a litter as consisting of one or more dead or live young. Some workers have been using the mean fertility dose as the dose denoting vitamin E "activity". Palmer ('37) expresses the results in per cent efficiency, not only of the live litter, but also of the total (live plus dead) litter, total implantation and live implantation. These efficiencies are defined, respectively, as the percentage of rats in a group producing live litters, producing live and dead litters, the percentage of total implantations resulting in live and dead young and those resulting in live young. There are certain advantages in using the implantation efficiencies in addition to the litter efficiencies which make this method preferable. The autopsy is conducted after parturition in both of these methods. Palmer ('37) distinguishes between live, dead and resorbed fetuses. Any response having a live implantation efficiency of 10% or more is considered a positive response. Mason ('39, '42) employs the formula: $\frac{W + N}{5}$ = "uterine index", where W represents the

[1] Paper no. 47, Journal Series, General Mills, Inc., Chemical Research Department.

weight of the uterine contents and N the number of viable young when two or more are present. Uterine index values of less than 0.35, 0.35 to 0.99 and 1.0 or greater are classed, respectively, as negative, subminimal and positive responses. Mason performs the autopsy on the sixteenth day of pregnancy.

Evans and Burr ('27 b) reported that 22% lard was usually sufficient to inactivate the vitamin E in wheat germ even when the latter was incorporated in the diet at a level of 2%. Macomber ('33) reported that rats fed a diet containing 20% lard and supplements of 0.5 gm. or more of wheat germ became sterile. The animals reproduced when lettuce was fed separately even though the diet contained 20% lard. Rancid fats have frequently been held responsible for the destruction of vitamin E as shown in animal studies. It was reported by Weber, Irwin and Steenbock ('39) that there was no destruction of vitamin E when the latter and rancid fats were fed separately, but that three times as much vitamin E was required when the diet was composed of rancid fat rather than fresh fat. The present study was made to investigate some of the factors influencing the bioassay of vitamin E.

<div align="center">EXPERIMENTAL</div>

The method of assay is similar to that described by Palmer ('37) except for a number of modifications made by this laboratory. Ten day old females and their dams are transferred to Palmer's ('37) vitamin E-deficient diet.[2] At 70 days of age the young females are placed in individual cages and daily vaginal smears are made, using the procedure described by Long and Evans ('22). Evans and Burr ('27 a), Palmer ('37) and Bacharach and Allchorne ('38) report a high rate of pseudopregnancies, sometimes as high as 20%. We have experienced a very low rate, seldom exceeding 2%. We attribute this to our close observation of the males and the discarding of all those whose breeding performances are questionable. We have noted that pseudopregnancies are most prevalent when the males are used too frequently. Any female not mat-

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[2] The vitamin E-deficient diet is kept under refrigeration between feedings.

ing within 2 weeks following the depletion period is discarded. All the tests are conducted with virgin rats, because our experience has been that the vitamin E requirement for production of the second litter is higher than it is for the first litter. Initial doses of the test material are fed on the first day of pregnancy. Dry samples are either incorporated in the vitamin E-deficient diet or fed per se. Supplements consisting of oil or ethyl laurate solutions are fed orally in doses of 0.10 ml. or less by means of a 0.25 ml. calibrated syringe. Supplements are fed at a rate of three doses of 0.1 ml. per day until the total dosage has been fed. Supplements in excess of 3.0 ml. are fed in doses of 0.10 ml. during the first 10 days of gestation, so that one-tenth of the total supplement is fed each day. The autopsy of the test animal is made on the twenty-first day of gestation from 12 to 24 hours prior to parturition. The autopsy consists of an examination of the reproductive organs and their contents to determine the number of live young, dead young, resorption sites and implantation sites.

The conclusions drawn in our bioassays are based on both the live litter and the live implantation efficiencies. A 50% live litter efficiency and a 20% live implantation efficiency are taken as the lowest average response of a group of seven rats indicating vitamin E activity.

Implantation rate

The number of implantation sites of 2528 vitamin E assay rats [3] were tabulated (table 1). Their responses may be divided into three groups: those of less than seven implantations; those of seven to fourteen (inclusive) implantations; and those of more than fourteen implantations. If the above-mentioned first and third groups are discarded, it will mean a loss of 3.4% of the rats. Compared with the percentage of animals discarded in bioassays of some of the other vitamins, 3.4% is a relatively low percentage of discards. It has been learned that responses falling within these two groups have a

[3] These rats were used in assaying a large variety of samples, such as wheat germ oil, wheat germ, etc.

great effect on the average response of the assay group. The elimination of these responses would decrease the risks of interpreting, for example, a response of two live young from a rat having a total of two implantations, as equivalent to a response of eleven live young from a total of eleven implantations. The same principle may also be applied to rats having responses of more than fourteen implantations. The questionable interpretation of these responses accounts for much of the error in some vitamin E assays and the failure to consider the implantation efficiency also increases the percentage of error.

TABLE 1

Showing the per cent distribution of implantation sites of 2528 vitamin E assay rats.

NO. OF IMPLANTATION SITES	NO. OF RATS	PER CENT OF TOTAL	NO. OF IMPLANTATION SITES	NO. OF RATS	PER CENT OF TOTAL
1	3	0.12	10	526	20.7
2	7	0.27	11	605	23.9
3	7	0.27	12	431	17.4
4	7	0.27	13	247	9.7
5	13	0.51	14	90	3.6
6	9	0.35	15	29	1.1
7	57	2.3	16	6	0.23
8	149	5.8	17	5	0.20
9	336	13.2	18	1	0.04

Dosing

Mason ('39) found that the procedure employed in the administration of vitamin E has a pronounced effect on the efficiency of the absorption or the utilization of the vitamin. Dosing prior to mating rather than dosing during pregnancy results in lower reproduction efficiency. Mason also found that single doses fed on the eighth day of pregnancy yielded the most satisfactory results. However, it is difficult to feed the total dosage of low potency samples in a single day, and furthermore it may influence the consumption of the basal diet. A group of tests (table 2) were run with whole wheat to determine a satisfactory procedure for assaying samples of a low vitamin E potency. The sample of whole wheat used in these

tests was previously found to be active at a level of 45 gm., when fed at a rate of 4.5 gm. per day during the first 10 days of pregnancy. The lessened effectiveness of dosing prior to mating is indicated by groups 2 and 3 and also by groups 4 and 5 (table 2).

A study was made of the effects of cod liver oil (U.S.P.), lard (commercially packaged lard), tapioca dextrin (auto-claved tapioca), enriched patent flour [4] and fat-free enriched patent flour [5] on the vitamin E activity of high- and low-potency wheat germ oils (table 3). These tests were all conducted by means of the experimental procedure previously

TABLE 2

Showing the effect on vitamin E assays when supplements of ground whole wheat are fed before and after mating.

GROUP NO.	RATS NO. OF	GRAMS OF WHEAT FED PER DAY	DAYS OF DOSING		LIVE LITTER EFFICIENCY	LIVE IMPLANTA-TION EFFICIENCY
			Before mating	After mating		
1	3	0	..	..	0	0
2	7	6	10	..	0	0
3	4	6	..	10	75	74
4	5	3	10	10	80	51
5	5	3	..	20	100	98

described. The lard and cod liver oil were withheld from the basal diet as indicated in table 3. The supplements of tapioca dextrin, enriched patent flour, and fat-free enriched patent flour were incorporated with the basal diet as indicated. The wheat germ oils and the fat extract of enriched patent flour were fed by means of a syringe. All the supplements were fed during the first 10 days of pregnancy at the rate of one-tenth of the total dosage per day. The regular basal diet was fed ad libitum to all rats from the tenth day of pregnancy until the time of autopsy.

In table 3, groups 2 and 3 correspond to the regular vitamin E assay of wheat germ oil with the exception of the longer

[4] The thiamine, niacin and iron contents per pound of enriched patent flour as specified in Federal Register 6, 2580, 1941 are 1.66 mg. to 2.5 mg., 6.0 mg. to 24 mg. and 0.6 mg. to 24 mg., respectively.

[5] The fat content of the enriched patent flour was 1.0%.

dosing period. Enriched patent flour showed no vitamin E activity when it was fed at the 60 gm. level, however groups 10 and 12 indicate that 30 gm. of the flour exert as great an effect on littering as 150 mg. of wheat germ oil. The influential effect of patent flour is appreciable as shown also by a comparison of the responses of groups 3 and 6. It is shown by the results of groups 3 and 19 and 2 and 15 that the presence of lard and cod liver oil in the basal diet interferes with the ab-

TABLE 3

Showing the effects of tapioca, enriched patent flour, fat-extracted patent flour, fat extract of enriched flour, lard and cod liver on the vitamin E potency of high- and low-potency wheat germ oils.

| | | | | DIETARY FACTORS TESTED | | | | | | | |
| | | | | | Amounts fed as supplements | | | | | | |
GROUP NUMBER	NUMBER OF RATS	Lard. in basal diet	Cod Liver Oil. in basal diet	Tapioca	Enriched patent flour	Fat-extracted enriched patent flour	Fat extract of enriched patent flour	High-potency wheat germ oil	Low-potency wheat germ oil	LIVE LITTER EFFICIENCY	LIVE IMPLANTATION EFFICIENCY
		%	%	gm.	gm.	gm	gm	mg.	mg.	%	%
1	8	22	2	0	0	0	0	0	0	0	0
2	7	22	2	0	0	0	0	0	500	14	13
3	6	22	2	0	0	0	0	500	0	67	40
4	6	22	2	0	60	0	0	0	0	0	0
5	7	22	2	0	60	0	0	0	500	57	28
6	6	22	2	0	60	0	0	500	0	100	80
7	7	22	2	60	0	0	0	0	500	57	28
8	7	22	2	0	0	59.4	0	0	500	57	31
9	7	22	2	0	0	0	0.6	0	500	43	7
10	6	22	2	0	60	0	0	350	0	100	51
11	6	22	2	0	60	0	0	200	0	33	5
12	6	22	2	0	30	0	0	500	0	83	51
13	6	22	2	0	30	0	0	350	0	67	17
14	6	22	2	0	30	0	0	200	0	0	0
15	7	0	0	0	0	0	0	0	500	71	48
16	7	11	1	0	0	0	0	0	500	71	33
17	7	0	2	0	0	0	0	500	0	71	39
18	7	22	0	0	0	0	0	500	0	71	24
19	7	0	0	0	0	0	0	500	0	100	81

sorption or utilization of vitamin E from wheat germ oil. The absence of either the lard or the cod liver oil does not improve the utilization of vitamin E; however, the absence of both or a 50% reduction of both in the basal diet improves the reproduction efficiency of the assay rats. It may be noted that the vitamin E-deficient diet consists of 35% tapioca dextrin and that a 60 gm. supplement of this dextrin exerts an effect on vitamin E utilization nearly similar to those of 60 gm. supplements of enriched patent flour and fat-extracted patent flour. It may likewise be noted (groups 6, 19 and 3) that 60 gm. of patent flour and the fat-free basal diet yielded results of 100% live litter efficiency and 80% and 81% live implantation efficiency, respectively, as compared with 67% live litter efficiency and 40% live implantation efficiency when the regular assay procedure was used. The fat intake is lowered from 75% to 80% when a 60 gm. supplement of patent flour or tapioca dextrin is fed over the 10 day period. Therefore, it appears that the increased vitamin E activity, as a result of feeding supplements of dextrin or patent flour, is due to the lowering of the intake of lard and cod liver oil.

SUMMARY

The methodology of the vitamin E bioassay has been studied and it has been noted that the assay results are improved by discarding rats having less than seven and more than fourteen implantations.

The lessened effectiveness of dosing prior to mating was demonstrated.

Autopsy of the assay rats prior to parturition eliminates possible errors due to infanticide.

The high rate of pseudopregnancies (10 to 20%) reported by other workers was not experienced.

The vitamin E activity of an assay sample is influenced by the presence of 22% lard and 2% cod liver oil in the basal diet during the dosing period. The use of a fat-free basal diet during the dosing period results in much higher live litter and live implantation efficiencies.

ACKNOWLEDGMENT

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THE PERFORMANCE OF NORMAL YOUNG MEN ON CONTROLLED THIAMINE INTAKES [1]

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TWO FIGURES

For our purposes we define the thiamine requirement of man to be the amount needed to allow maximal and most efficient performance of the body. Practical application, however, requires further definition. What functions and characteristics of the body should we measure?

Outstanding characteristics of definite thiamine deficiency are weakness, fatigue, neuro-muscular incoordination, anorexia and disturbances of carbohydrate metabolism. With the exception of anorexia these characteristics are capable of quantitative evaluation under controlled conditions. Such measurements under rigidly standardized performance test conditions should allow estimation of the level at which thiamine restriction does, in fact, affect the functional capacity and efficiency of the organism. This approach was applied in the studies reported here. In addition urinary excretion and subjective reports were examined.

We have emphasized the following: (1) ability to do brief severe work of the type that is largely anaerobic; (2) ability to do moderately prolonged work of a severity close to the maximum at which a relatively steady state can be maintained; (3) ability to carry out various tasks of psychomotor function involving speed and coordination; and (4) ability to maintain normal carbohydrate metabolism, including its intermediary details.

<div align="center">SUBJECTS</div>

All subjects were men students between the ages of 18 and 30 years, free from signs and history of significant abnormality. None had peculiar habits of diet or activity. They were selected for personal reliability and were under daily observation but were not confined except at mealtimes and for 1 day each week. All were physically active but none was engaged in regular team sports. Outside eating was strictly forbidden except on rare occasions by special arrangement. A very small number of lapses occurred and were reported; these proved to be unimportant.

<div align="center">DIET</div>

All meals were prepared in the diet kitchen of the Laboratory. Care was taken to make the food as attractive and palatable as possible. Every portion was weighed and an extra identical meal was put aside for analysis at each meal time. In the first two series these meals were ground, quick-dried in a drier at 40–45° C., and then stored at –25° C. until analyzed. This procedure proved to allow significant losses of thiamine so thereafter the meals were ground and either analyzed immediately or quick-frozen and stored at –25° C. for analysis. The thiamine content of the diet for each day was also calculated from tables assembled from the literature.

The basal diet contained 340 to 380 gm. of carbohydrate, 100 to 120 gm. of protein, 110 to 130 gm. of fat and furnished 3,000 Cal. per day. Slight deviations, ± 200 Cal., sufficed to maintain body weight almost exactly constant in all subjects.

Each subject received a daily supplement of 2 mg. riboflavin, 25 mg. nicotinic acid amide and 50 mg. of ascorbic acid.

In series I and II half of the subjects received an additional 1 mg. of thiamine daily while the other subjects received placebos. The two groups were reversed in the second half of these experimental periods. At the end of experimental series III and IV each subject was given 4.11 mg. thiamine chloride by mouth and the excretion of thiamine was measured in the urine for the succeeding 24 hours.

ANALYTICAL METHODS

The 24 hour urine sample was collected in a glass bottle containing 5 cc. of glacial acetic acid and 5 cc. of toluol. The samples were stored at 5° C. for not more than 24 hours after collection. Thiamine was estimated by the thiochrome method of Hennessy and Cerecedo ('39) as modified by the Research Corporation Committee for the determination of thiamine in cereal products (privately circulated, '41). The same thiamine method was applied to the foods. With both foods and urine, tests of recovery of added thiamine were made.

Venous blood, drawn with a minimum of stasis, was analyzed for lactate by the method of Friedemann and Cotonio as modified by Edwards ('38). Pyruvate was estimated in the blood by the method of Lu ('39) as modified by Friedemann and Haugen ('43).

Blood sugar was measured by the method of Folin and Wu. Hemoglobin was measured as oxyhemoglobin in the photoelectric colorimeter. Blood ketones were estimated in series I by the method of Barnes and Wick ('39).

PSYCHOMOTOR TEST METHODS

The following tests were used as a "battery" covering: (1) manual speed and coordination, (2) motor control, and (3) velocity of small hand movements. They were designed so they could be carried out during actual work on the treadmill. The three tests were given in consecutive order of manual speed, motor control and tapping, with a rest of 30 seconds

between each test. The duration of the manual speed and motor control tests was 60 seconds each; the tapping test lasted 30 seconds in which the rates were measured for each 10 seconds.

Manual speed (Brozek, '43) was tested with an open pipe 30 cm. long, and 1.3 cm. internal diameter, which was held vertically by the left hand. The task was to place a steel ball, 1.3 cm. in diameter, in the top of the pipe, catch it at the bottom and repeat the cycle as fast as possible. A mechanical counter recorded the number of times the ball passed through the pipe. A cloth bag attached by a wire hoop at the bottom of the pipe served to stop the balls not caught.

Motor control was tested by tracing a winding path 1.1 cm. wide and 0.6 cm. deep with a stylus 0.5 cm. in diameter. The path was 500 cm. long arranged in a field 48 by 35 cm., and provided a variety of movement patterns (short and long, angular and circular, proceeding right and left). The number of contacts and the total duration of contacts between the stylus and the side walls of the path were recorded electrically and served as performance criteria.

Velocity of small hand movements was tested by tapping, as rapidly as possible, 2 brass plates 4.8 cm. in diameter and placed 1.7 cm. apart. The plates were separated by a wooden barrier 1 cm. high and were tapped alternately (to eliminate tremor tapping). The number of taps was recorded electrically in the first and the third 10-second intervals of a 30-second work period.

EXPERIMENTAL PROGRAM

There were four series of experiments each lasting 10 to 12 weeks exclusive of the preliminary period of training and standardization. There were four subjects in each series; three of the subjects took part in three series each so that there was a total of ten subjects and extensive control data were accumulated on three of the men. The average thiamine intakes in the four series (I, II, III, IV) were, respectively, 0.63, 0.53, 0.33 and 0.23 mg. per 1,000 total Calories.

During the first half of series I and II half of the subjects received a daily supplement of 1 mg. of thiamine while the other men received placebos. During the second half of these series the order was reversed so that the men formerly on placebos now received the thiamine supplement and vice versa. None of the men knew either of these arrangements or of the contents of the other tablets (ascorbic acid, riboflavin and niacin) which all men received daily in all experiments.

In all four series the general arrangement of tests was the same. Once a week each man was studied, in the basal postabsorptive state, in a half-day series of tests in a constant environment of 78° F. and about 50% relative saturation. The subject was dressed in shoes, shorts and undervest and returned to bed for 20 to 30 minutes before a blood sample was taken. The cardiotachometer was then attached and the standing heart rate was recorded for 3 minutes before these psychomotor tests were started. At the end of these experiments, work was started on the motor-driven treadmill. The first exercise test consisted of walking at a rate of 3.85 miles per hour at a 12% rate of climb. This means accomplishment of external work at a rate of 870 kg. meters per minute by a man weighing 70 kg., and required an oxygen usage of about 2730 cc. per minute and an expenditure of about 820 calories per hour. In series I this work period was 90 minutes; the work period was 60 minutes in series II, III and IV. The heart rate was recorded during work and during recovery except while psychomotor tests were in progress. The battery of psychomotor tests was run through once in the first 15 minutes of work, again in the last 15 minutes of work and finally in recovery starting 5 minutes after the end of work.

In series II, III, and IV this first exercise test was followed, after 10 minutes for partial recovery, by 2 minutes of running at 7 miles per hour at a 10% rate of climb (1316 kg. meters per minute for a 70 kg. man).

Every second week blood samples were drawn at 3 and at 10 minute intervals after walking and at 5 and 15 minutes after running. Twenty-five minutes after running 100 gm. of glu-

cose were ingested and further blood samples were then taken at intervals of 30, 60, 90 and 120 minutes while the subject rested quietly.

Roentgenograms of the heart and electrocardiograms were made on each subject at the start and at the end of the restricted thiamine periods.

Thiamine was measured in the 24 hour urine in all except series I. At the end of series III and IV a thiamine saturation test was made on the day following the last 24 hour urine collection.

RESULTS

The data collected are so extensive that they can only be presented as averages and examples.

General observations and subjective reports

Apart from the initial adjustments to the rigid regime and some slight training effects, both personal characteristics and performance appeared to be remarkably constant in all subjects in all four series. The close daily contacts between subjects and observers gave ample opportunity to observe changes in behavior and apparent ability to carry out the several tests. Muscle hyperesthesia or calf tenderness did not occur in spite of the weekly periods of severe walking and running exercise. No obvious changes were seen. We were fortunate that neither illness nor emotional crises occurred at any time.

The subjects were questioned for subjective reports from time to time. Care was taken to avoid suggestive questions. The normal variations in vigor and sense of well-being were recorded, these oscillations being neither unusually frequent nor intense. There was occasional worry over college examinations, a few mild states of the ''blues'' and a complete absence of any trend or consistency in these subjective states. Certainly all general observations and subjective reports would suggest that it was a matter of complete indifference whether the men received 0.23 or 0.96 mg. of thiamine per 1,000 Calories.

Heart sizes and electrocardiograms

Heart sizes were measured in systole and diastole by the roentgenkymographic method (Keys et al., '40). No changes beyond the error of the method occurred at any time in any subject. The electrocardiograms (Leads I, II, and III) were likewise entirely negative. The points examined were: (1) Axis deviation, (2) P-Q interval, (3) Q-S interval, (4) voltage of R, (5) voltage of T, (6) regularity.

Heart rates

Table 1 shows that the heart rates while standing, during work and in recovery were unaffected by thiamine intakes of 0.53 and of 0.96 mg. per 1,000 Cal. Table 2 shows that sub-

TABLE 1

Heart rates in rest (standing), in the last minute of 90 (series I) or 60 (series II) minutes of severe work, and for the second 60 seconds of recovery. Averages, in beats per minute, for all subjects for all tests in the periods on the designated thiamine intakes; these last are in milligram per 1000 Cal.

PERIOD	THIAMINE			
	Series I		Series II	
	0.63	0.96	0 53	0.86
Standing rest	93	96	89	88
Last minute work	152	153	151	158
Second minute recovery	114	116	127	133

TABLE 2

Heart rates in rest (standing), in the last minute of 60 minutes of severe work and for the second minute of recovery. Averages, in beats per minute, for all subjects for the weeks after the start of thiamine restriction. Series III — 0.33 mg. per 1000 Cal.; series IV — 0.23 mg. per 1000 Cal.

PERIOD	WEEK					
	1	2	3	5	7	9
Series III						
Rest	90	...	95	91	99	91
Work	152	...	155	151	144	144
Recovery	130	...	126	130	126	118
Series IV						
Rest	89	86	...	95	88	97
Work	152	147	...	144	150	149
Recovery	126	121	...	119	124	128

sistence on thiamine intakes of 0.33 and of 0.23 mg. per 1,000 Cal. produces no progressive trend in the heart rates in either rest, severe work or in recovery.

In series I and II the blood lactate and pyruvate values were substantially identical at all times in the groups on the "high" as compared with the "low" thiamine intakes and this was true after work as well as in rest. Table 3 shows the averages for series III and series IV which indicate that the

TABLE 3

Lactate and pyruvate concentrations, in milligrams per 100 cc. blood, in series III and IV during subsistence on 0.33 and 0.23 mg. thiamine per 1000 Cal., respectively. Values in rest (Rest), at 10 minutes after 60 minutes of work (Work 2), at 5 minutes (Work 3) and at 15 minutes (Work 4) after 2 minutes of extreme work, and at 60 minutes after ingestion of 100 gm. of glucose (Tol. 2). Values tabulated are averages for all subjects for the weeks after start of the thiamine restriction. L = lactate, P = pyruvate.

SERIES AND TIME	1 WEEK		2 WEEKS		3 WEEKS		5 WEEKS		7 WEEKS		9 WEEKS	
	L	P	L	P	L	P	L	P	L	P	L	P
Series III												
Rest	15.6	2.02	...	...	16.4	1.91	14.9	1.98	18.8	2.04	13.6	1.60
Work 2	11.1	...	...	...	14.2	...	11.1	...	10.9	...	10.4	...
Work 3	58.2	3.50	...	...	62.0	3.35	63.7	3.30	56.2	3.18	43.9	3.18
Work 4	45.4	2.97	...	...	54.9	3.29	43.1	3.03	52.1	2.79	39.4	2.79
Series IV												
Rest	...	...	13.6	1.54	15.1	1.56	14.9	1.68	13.9	1.83	13.6	1.59
Work 2	...	...	15.8	...	19.6	...	14.5	...	20.7	...	19.0	...
Work 3	...	...	51.7	2.98	55.5	2.78	44.4	3.12	58.7	2.87	52.1	3.07
Work 4	...	...	45.6	2.81	51.2	2.67	44.7	3.03	64.6	2.87	46.8	2.84
Tol. 2	...	...	9.7	1.43	12.1	1.26	13.4	1.50	15.0	1.55	13.8	1.35

blood levels of these metabolites are unaffected in any consistent manner by thiamine intakes of 0.33 and of 0.23 mg. per 1,000 Cal. Further comparison may be made with the four subjects in series II who received from 0.53 to 0.86 mg. thiamine per 1,000 Cal. At the end of 10 weeks these "controls" averaged 1.79 mg. pyruvate per 100 cc. of blood in rest, 3.27 mg. for "Work 3," and 3.15 mg. for "Work 4" ("Work 2" blood was not drawn in the earlier series). It is notable that there was actually a fall in the pyruvate following glucose adminis-

tration (cf. Williams et al., '43). It is evident that in these young men the smallest thiamine intake used— 0.23 mg. per 1,000 Cal. — was sufficient to allow intermediary carbohydrate metabolism to proceed independently of the thiamine intake.

Blood sugar

Tables 4 and 5 give the average blood sugar values for the four series in rest, after work, and in glucose tolerance tests. From table 4 it appears that thiamine intakes higher than 0.63 or 0.53 mg. per 1,000 Cal. may be associated with slightly higher blood sugar levels at all these times. The significance

TABLE 4

Glucose concentrations, in milligrams per 100 cc. of blood, in rest (Rest), *at 3 minutes* (Work 1) *and at 10 minutes* (Work 2) *after 60 or 90 minutes walking, at 5 minutes* (Work 3) *and at 15 minutes* (Work 4) *after 2 minutes of extreme exertion and at 30, 60, 90 and 120 minutes after ingestion of 100 gm. of glucose* (Tol. 1, Tol. 2, Tol. 3, *and* Tol. 4, *respectively*).

THIAMINE INTAKE	REST	WORK 1	WORK 2	WORK 3	WORK 4	TOL. 1	TOL. 2	TOL. 3	TOL. 4
Series I, 0.63	77.8	74.2	76.8	..	...	135.5	154.2	134.9	119.7
Series I, 0.96	80.8	77.1	80.1	..	...	139.8	157.7	138.1	121.1
Series II, 0.53	79.2	79.1	80.9	81.7	78.2	140.7	145.6	123.9	....
Series II, 0.86	84.9	83.9	85.6	85.4	80.3	149.0	145.7	129.6	....

of this is uncertain though the relation is consistent and is not due to methodological error. However, maintenance at thiamine intakes of 0.33 or 0.23 mg. per 1,000 Cal. does not result in any tendency for the blood sugar levels or their regulation to change in any consistent fashion.

Psychomotor tests

There were steady but small increases from week to week in all the psychomotor test scores under all conditions. Presentation of the voluminous data from the psychomotor tests is hardly justified in view of the fact that statistical analysis of the scores in all three tests before, during, and after work showed only the same slight but steady improvement from

week to week, regardless of the thiamine intake. This was true for each of the test subjects as well as for the averages for all. The control data indicate that the rate of this gradual improvement was normal in the men on the restricted thiamine intake. Illustrative results for motor control are given in figure 1.

The psychomotor tests used here are as sensitive as any of which we have knowledge. It must be concluded then that re-

TABLE 5

Glucose concentrations, in milligrams per 100 cc. of blood, in series III and IV during subsistence on 0.33 and 0.23 mg. thiamine, respectively. Values in rest (Rest), at 3 minutes (Work 1) and at 10 minutes (Work 2) after 60 minutes of work, at 5 minutes (Work 3) and at 15 minutes (Work 4) after 2 minutes of extreme work, and at 30, 60 and 90 minutes after ingestion of 100 gm. of glucose (Tol. 1, Tol. 2, and Tol. 3, respectively). Values tabulated are averages for all subjects for the weeks after start of the thiamine restriction.

PERIOD	REST	WORK 1	WORK 2	WORK 3	WORK 4	TOL. 1	TOL. 2	TOL. 3
Series III								
1 week	86.9	69.6	70.3	78.2	77.0	124.9	107.4	105.4
3 weeks	81.0	70.4	79.9	80.6	79.8	113.1	113.7	97.4
5 weeks	86.3	75.8	80.4	82.8	79.5	128.4	133.1	117.5
7 weeks	77.4	71.9	76.8	74.3	69.2	117.5	104.0	90.0
9 weeks	84.7	73.9	75.6	72.1	68.1	122.6	114.0	108.5
Series IV								
2 weeks	...	...	79.8	80.9	78.6	137.2	139.5	125.3
3 weeks	...	...	81.3	80.5	80.6	121.7	136.0	124.8
5 weeks	...	...	80.3	78.0	80.2	134.3	129.3	101.1
7 weeks	...	...	79.5	77.1	77.6	137.5	121.0	117.3
9 weeks	...	...	93.0	86.9	84.6	128.1	124.0	109.1

striction of these normal young men to 0.23 mg. of thiamine per 1,000 Cal. had no significant effect on these typical measures of psychomotor speed, coordination and accuracy, whether measured in rest or in work.

Miscellaneous observations

In series I blood ketones were measured before and after work. The changes induced by the work were small and the results on the two different levels of thiamine intake disclosed no differences as large as the experimental error.

Blood hemoglobin, estimated every second week in all series, was remarkably constant on the average. In series I and II the average values were almost identical for the periods on low and high thiamine intake. In series III there was a tendency toward a slight rise in hemoconcentration as time went on (averages for first 3 weeks, 14.55; last 3 weeks, 15.00 gm. per 100 cc.).

Strength tests with grip, leg, and back dynamometers showed nothing but gradual and irregular training effects unrelated to the thiamine intake.
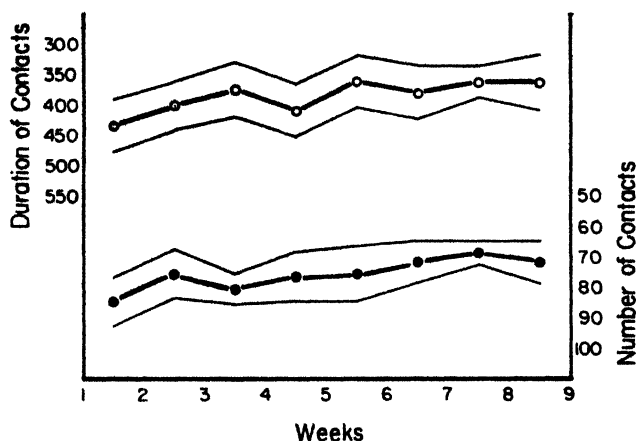
Fig. 1   Motor control in series IV during the first work period each week. The areas between the outer lines for duration of contacts and number of contacts enclose the standard deviations of the average scores plotted. High scores indicate poor performance.

Thiamine excretion

Thiamine excretion was not measured in series I. In series II the average 24-hour excretion of thiamine in the fourth and fifth weeks was 101 µg. on 1.59 mg. per day (0.53/1000 Cal.) and 291 µg. on 2.59 mg. per day (0.86/1000 Cal.).

In series III the 24-hour thiamine excretion averaged 106 µg. in the second week on 1.0 mg. per day intake (0.33 mg. per 1,000 Cal.) and remained at about that general level throughout; at the tenth week the average output was 92 µg. Individual

variations in single measurements varied from 38 to 151 µg. but the averages for the different individuals were fairly constant; for the last 8 weeks the individual average excretions were: 89, 107, 80 and 90 µg. per 24 hours. Saturation tests at the end with 4.11 mg. of thiamine chloride (orally) gave 7 to 10% recoveries except in a test on one man in which 26% of the test dose was excreted.

Figure 2 shows the average intakes and excretions of thiamine in series IV. Saturation tests on the subjects in series IV were made at the end of the experiment. The average recovery of the test dose was 6.4%.
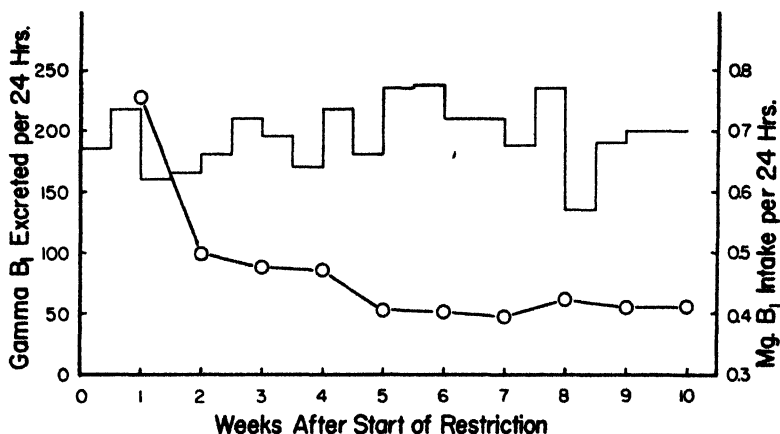
Fig. 2   Average 24-hour urinary excretion and average daily intake of thiamine in series IV. Excretion shown by circles.

DISCUSSION

The thiamine intakes listed in this paper are in terms of thiochrome analyses. These averaged 10.3% (standard deviation ± 6.7%) more than the values calculated from the most recent tables (Bowes and Church, '40; Booher et al., '42; Cheldelin and Williams, '42). Checks with meat samples by the rat growth method likewise indicated that the thiochrome method as used here slightly overestimates the true thiamine values. Accordingly we can say that the intakes were not more than stated and may have been 5 to 10% less.

The pyruvate values in the present experiments are of particular interest in view of the belief that an unusual rise in blood pyruvate in exercise (Lu and Platt, '39) or after glucose ingestion (Williams et al., '40, '41, '42, '43) is a specially sensiitve indicator of "hidden" or "latent" thiamine deficiency. It will be noted that the Mayo Clinic group found the maximum effect in their deficient subjects to be at 60 minutes after glucose. In our subjects at this time the blood pyruvate was uniformly lower than during rest on 0.23 mg. of thiamine per 1,000 Cal.

So far as comparisons can be made it appears that the per cent of dietary thiamine excreted in the urine was in reasonable agreement with expectations from other work (cf. Melnick, Field and Robinson, '39; Jolliffe et al., '39; Carden, Province and Ferrebee, '40; Williams et al., '42, '43). These comparisons raise a question: Should we consider the absolute amount of thiamine excretion or should we emphasize the proportion of dietary thiamine excreted? This may be important in comparing our subjects, who lived at a level of slightly over 3,000 Cal. per day, with those of Williams et al. ('43), who were maintained on a much lower level. In any case it appears that the excretions in series IV were in the range generally considered "deficient" (cf., e.g., Wang and Yudkin, '40; Elsom et al., '42; Melnick, '42; Melnick and Field, '42).

Of all the functions and characteristics measured in these studies the only evidence of any deficiency was in the urinary excretions. However, many authorities would consider the thiamine intake grossly deficient in series IV and at least moderately deficient in series III.

Estimation of the thiamine requirement demands definition of the bases on which the "requirement" is assessed. Most estimates for man have relied on one or more of the following criteria: (1) the appearance or removal of various signs or symptoms of disease; (2) the excretion of thiamine with or without a "saturation" test dose of the vitamin; (3) analogy with the results of animal studies, particularly growth rates.

The problem of the thiamine requirement of man is no longer chiefly concerned about the amount needed to prevent or to cure beri-beri. The "sub-clinical deficiency states" are, by definition, characterized by the absence of well-marked signs and symptoms. Vague subjective complaints and psychological alterations do not lend themselves to measurement and adequate control.

Thiamine excretion is undoubtedly related to the thiamine intake and "stores" in the body and this measurement is useful for many purposes. However, true adequacy or deficiency is not indicated by this means except by special definition. The difficulty is illustrated by the difference in interpretation of excretion levels by Wang and Yudkin ('40) and Williams et al. ('43), on the one hand, and Holt ('43) on the other.

There is now ample evidence that different animal species vary widely in thiamine requirements. Computations for man from experiments on the dog or rat may be grossly erroneous.

The conclusions to be drawn from any set of experiments are always somewhat limited to the conditions and materials of the experiment. This limitation is unusually narrow in vitamin studies at present because of the large number of factors which may play a role. Age, sex, climate, metabolic activity, disease and other foodstuffs may all contribute to the quantitative result. The duration of the period of subsistence is undoubtedly of importance.

The limitations in the present experiments are clearly recognized. We can conclude only that normal healthy young men living at around 3,000 Cal. per day on an ordinary balance of foodstuffs with an adequate supply of other vitamins are not benefitted, in any demonstrable way, by a thiamine intake of more than 0.23 mg. per 1,000 Cal. over a period of some 3 months. The possibility was not ruled out that less than 0.23 mg. would suffice or that deficiency might eventually appear on the 0.23 mg. regime.

In previous studies (Keys and Henschel, '42) we could find no physiological reason for increasing the thiamine intake of soldiers above the garrison ration level of about 0.43 mg. per

1,000 Cal. This finding was confirmed by Simonson and Enzer ('42) and was further confirmed on a broader basis in series I and II reported here.

SUMMARY AND CONCLUSIONS

1. Thiamine intake was limited and measured in four series of experiments. Each experiment lasted 10 to 12 weeks and involved four normal young men subjects. The diet was adequate except for thiamine and balance was maintained at 3050 ± 200 Cal. per day. Two control series were run in which all conditions and tests were applied identically but the subjects received in addition 1 mg. of thiamine daily.

2. Average thiamine intakes studied were, in milligram per 1,000 Cal., 0.63, 0.53, 0.33, and 0.23 as determined by thiochrome analyses on all food. Calculated values were 10.3%, $c = \pm 6.7\%$, less than the thiochrome analyses.

3. Excretion of thiamine in the urine averaged, after a few weeks, about 10% of the dietary thiamine on an intake of 0.33 mg. per 1,000 Cal., and about 7% on 0.23 mg. Saturation tests gave similar results.

4. All subjects adhered to a fixed regime of weekly work performance. Blood and other examinations were made at regular intervals. All conditions and experiments were rigidly standardized. Tests covered simple strength, responses during brief exhausting work, prolonged severe work, and in recovery, psychomotor tests of speed and coordination, and glucose tolerance.

5. General and clinical observations and subjective reports were analyzed. Electrocardiographic and roentgenographic studies were made. Variables measured in rest, work, and recovery included heart rates, blood pyruvate, lactate, glucose and hemoglobin, and the psychomotor performances.

6. All results are in agreement that, for the periods studied, no benefit of any kind was observed to be produced by an intake of more than 0.23 mg. of thiamine per 1,000 Cal. At this level of intake muscular, neuromuscular, cardiovascular, psychomotor and metabolic functions were in no way limited by the

thiamine restriction. Clinical signs, subjective sensations and state of mind, and behavior were likewise apparently unaffected by thiamine intakes from 0.96 to 0.23 mg. per 1,000 Cal.

7. From the present work no conclusions are drawn as to requirements for men during more prolonged periods, or for women, children, the aged, or for states of disease or injury.

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STUDIES OF THE AVERAGE AMERICAN DIET

II. RIBOFLAVIN, NICOTINIC ACID AND PANTOTHENIC ACID CONTENT

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The rapid accumulation of knowledge regarding the B vitamin contents of foods has made it possible to evaluate more properly than was previously possible the actual amount of these foods entering into the average American diet. These studies are especially timely at present in view of their importance to national nutrition.

Such an appraisal has already been made for thiamine (Lane, Johnson and Williams, '42). The present paper represents an extension of the above work, including the vitamins riboflavin, nicotinic acid and pantothenic acid. [2]

SELECTION OF FOODS

As in the thiamine studies (Lane, Johnson and Williams, '42), we have included assays of the more important foodstuffs in the American diet in proportions corresponding to the annual per capita consumption of each in the period 1934–1937. [3] Due to the increased scope of the present work

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[2] The work here presented is part of a study originally undertaken in the private laboratory of R. R. Williams, Summit, New Jersey, but which due to war interruptions was concluded at the University of Texas. The plan of the first paper of this series (Lane, Johnson and Williams, '42) has been followed in this one.

[3] This dietary does not reflect changes incident to the war period among which is the progressive enrichment of bread and flour.

the assays of mixed diets, as reported for thiamine, have been omitted; only assays of individual foods are reported here.

EXPERIMENTAL

The methods of sampling foods, preparing extracts and assaying for the various B vitamins have been described previously in detail (Cheldelin, Woods and Williams, '43; Cheldelin and Williams, '42).

Riboflavin, nicotinic acid and pantothenic acid content of individual foodstuffs

Tables 1–4 contain assays of all articles of foods studied, for each of the three vitamins listed above.

Wherever replicate samples have been assayed, either in this or previous papers (Cheldelin and Williams, '42; Cheldelin, Woods and Williams, '43) the values in tables 1–4 represent the average of the values obtained. The individual assays were obtained in many cases upon samples of different varieties and from different locations, in order to minimize differences in vitamin content due to these factors. Such environmental or varietal differences may sometimes be significant but are usually not great, as may be seen from an examination of the riboflavin assay values in table 5. Similar results have been obtained for thiamine and riboflavin in grains (Conner and Straub, '41; Nordgren and Andrews, '41).

Cooking waters and meat juices have been included as part of the cooked foods assayed except in a few cases where the cooking waters were assayed separately and discarded. The values in tables 1–4 therefore tend to represent maximum amounts of the various vitamins present after cooking.

Principal contributors of riboflavin, nicotinic acid and pantothenic acid to the average American diet

Riboflavin. As was pointed out for thiamine (Lane, Johnson and Williams, '42) the wide variations in caloric contributions of different foods to diets often cause the total vita-

min contributions of these foods to be quite different than would be expected on the basis of relative vitamin contents alone. Thus white bread, even though a relatively poor source of riboflavin, is second only to milk as a total contributor of riboflavin to the diet. Eggs are third in importance, and potatoes are a poor fourth. Liver is fifth in order despite its relatively high content of riboflavin. Dairy products as a class contribute nearly half of the total riboflavin supply to the diet, meats supply about one-fifth, and cereals, as well as fresh fruits and vegetables, each furnish about one-sixth of the total.

If the white bread in the diet were enriched to 0.7 mg. per pound (equal to 1.54 µg. per gram) as provided by Food Distribution Order No. 1 which is now in effect, the total supply of riboflavin in the diet would be 1.61 mg. per 2500 calories, representing slightly less than a 12% increase in the present intake. This is much less substantial than is the case for thiamine (Lane, Johnson and Williams, '42) or for nicotinic acid, as indicated below.

The total calculated daily intake of 1.4 mg. of riboflavin is in fair agreement with the estimate of approximately 1.7 mg. by Sherman ('42). These values, on the other hand, are appreciably lower than estimates of normal human requirement (Elvehjem, '41; Sebrell, Butler, Wooley and Isbell, '41; Williams, '42). If these estimates are correct, it seems necessary that we should either greatly increase the use of dairy products, in order to make the riboflavin supply adequate, or resort to a relatively heavy fortification of some staple with riboflavin.

Nicotinic acid. Flesh foods as a class contribute approximately three-fifths of the total daily intake of 11 mg. of nicotinic acid. This large amount is considerably out of proportion to the fraction of the total of other B vitamins supplied by flesh foods. The individual important contributors are beef, pork and fish, which together furnish about two-fifths of the total nicotinic acid consumed. White bread, which furnishes

TABLE 1

Yield of riboflavin per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	RIBOFLAVIN Raw edible portion	Cooked edible portion	Total loss[2]	Yield of riboflavin in μg. Per 100 gm. raw edible portion[4]	Per 100 gm. as purchased[4]	REMARKS
	%[1]		% E. P.[1]	μg./gm.	μg /gm.	%			
Cereal products									
White bread	100	None	..		0.90			90	Approx. 2% milk solids
Rye bread	100	None	..		0.72			72	So labeled; no milk
Whole wheat bread (100%)	100	None	..		1.1			110	So labeled; no milk
Whole wheat bread	100	None	..		0.67			67	So labeled; no milk
Corn meal	100	Mush	+370		0.13			63	
Oatmeal	100	Porridge	+300		0.27			110	
Rice, white	100	Steamed	+200		0.20			60	Cooking water absorbed
Rice Krispies	100	None	..		0.47		47	47	
Dairy products									
Milk	100	None	..	1.7				170	Raw, commercial sample
Milk	100	Heated in glass dish	0	1.7	0.87	48	87	87	Raw, commercial sample
Eggs	86	Scrambled	−11	4.4	3.2	35	290	260	60–65 gm./egg
Eggs	86	Poached	0	4.4	4.4	0	440	380	60–65 gm./egg
Cheese, American	100	None	..	5.9			590	590	
Meats									
Beef									
Round	96	Fried	−25	2.2	2.3	22	170	160	Juices included
Liver	100	Fried	−13	30	35	0	3000	3000	Juices included
Heart	100	Steamed	−13	8.9	10	0	890	890	Cooking water used
Pork									
Bacon	100	Fried	−50	3.1	5.6	9	280	280	Fat discarded
Ham	84	Fried	−42	2.6	3.6	19	210	180	Fat discarded
Loin chops	80	Fried	−37	2.3	2.7	26	170	140	Juices included
Mutton									
Shoulder	75	Steamed	0	2.5	2.5	0	250	190	Cooking water used
Veal									
Chop	80	Fried	−29	1.4	2.0	0	140	110	Juices included
Chop	80	Steamed	0	1.4	1.4	0	140	110	Cooking water used
Lamb									
Leg	80	Roasted	−20	2.5	3.1	0	250	200	Juices included
Chicken									
Leg	75	Fried	−27	2.6	3.5	3	250	190	Juices included
Breast	75	Fried	−7	1.3	1.4	0	130	100	Juices included
Fish									
Salmon	100	Fried	−13	1.4	1.7	0	140	140	Juices included
Salmon, canned	100	None	..		1.5		150	150	Can liquor used
Halibut	100	Steamed	0	0.44	0.44	0	44	44	Cooking water used
Halibut	100	Fried	−17	0.87	1.0	22	68	68	Juices used
Vegetables, cooked									
Asparagus, canned	100	None	..		1.0	0	100	100	Can liquor used
Beans, canned	100	None	..		0.38	0	38	38	Can liquor used
Beans, lima	100	Steamed	..	1.3	1.3	0	130	130	Cooking water used
Beans, snap, canned	90	None	..		0.52	0	52	47	Can liquor used
Beets	55	Boiled, covered kettle	−7	0.38	0.34	18[5]	31	17	Tops removed. Cooked. (H_2O discarded)
							38	21	
Beets	55	Steamed	0	0.61	0.61	0	61	61	Cooked. H_2O used
Beet greens	100	Steamed	0	2.2	1.9	14	190	190	Cooking water used
Cabbage	80	Steamed	0	0.57	0.48	16	48	38	Cooking water used
Cauliflower	60	Steamed	0	0.61	0.52	15	52	31	Cooking water used
Corn, canned	100	None	..		0.80		80	80	Can liquor used
Okra	88	Steamed	0	1.0	1.0	0	100	88	Cooking water used
Onions	94	Fried	−77	0.24	1.0	26	18	17	
Peas, canned	100	None	0		1.0	0	100	100	Can liquor used
Peas, dried	100	Boiled	+200	1.8	0.60	0	180	180	Cooking water absorbed
Potatoes	85	Boiled, covered dish	+15	0.50	0.25	43[5]	28	24	Cooking water discarded
						0	50	43	Cooking water used
Potatoes	85	Steamed	+5	0.50	0.42	12[5]	44	37	Cooking water discarded
						7	47	40	Cooking water used
Pumpkin, canned	100	None	..		0.62		62	62	
Sauerkraut	100	Steamed	0	0.40	0.38	5	38	38	Cooking water used
Spinach	83	Steamed	0	2.2	1.6	27	160	130	Cooking water used
Sweet potatoes	100	Baked	−23	0.41	0.46	13	36	36	Skins used
Tomatoes, canned	100	None	..		0.32	0	32	32	Can liquor used
Turnips	66	Boiled, covered dish	+3	0.42	0.38	10[5]	38	25	Cooking water discarded
						0	42	28	Cooking water used
Vegetables, raw									
Cabbage	80			0.57			57	46	
Carrots	63			0.50			50	32	
Lettuce	95			0.27			27	26	
Tomatoes	97			0.37			37	36	
Fruits, cooked									
Apples	80	Boiled, open dish	−33	0.17	0.19	25	13	10	Made into applesauce, including liquor
Pears, canned	100	None	..		0.13		13	13	Can liquor used
Prunes, canned	97	None	..		0.25		25	24	Can liquor used
Prunes, dried	83	None	..				54	45	
Fruits, raw									
Apples	91			0.17			17	15	
Bananas	68			0.56			56	38	
Canteloup	50			0.26			26	13	
Grapefruit	66			0.30			30	20	
Oranges	63			0.47			47	30	
Peaches, frozen	100			0.17			17	17	
Raisins	100			0.29			29	29	
Strawberries	97			0.34			34	33	
Watermelon	59			0.69			69	34	
Miscellaneous									
Peanuts	69			1.1			110	76	
Chocolate	100			2.4			240	240	
Molasses	100			0.62			62	62	

[1] Per cent of "as purchased weight" after discarding inedible portions such as bones, tops, peelings, etc.
[2] Calculated to represent the change in weight of edible portion only. The values are only approximate when the inedible portion is discarded after cooking, as with bones in roasts, cobs from sweet corn, or skins from potatoes baked whole.
[2] Total loss does not include vitamin loss in cooking liquors specified under footnote 5.
[4] Includes vitamin in cooking waters unless otherwise indicated in "remarks" column.
[5] Total loss includes thermal loss and loss in cooking liquors.

TABLE 2
Yield of nicotinic acid per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	Raw edible portion	Cooked edible portion	Total loss[3]	Per 100 gm raw edible portion[4]	Per 100 gm as purchased[4]	REMARKS
	%[1]		%[ ] E.P.[2]	μg/gm	μg/gm	%			
Cereal products									
White bread	100	None	..		6.0 (7.0)[6]			600 (700)[6]	Approx. 2% milk solids
Rye bread	100	None	..		9.2 (11)			920 (1100)	So labeled; no milk
Whole wheat bread (100%)	100	None	..		28 (40)			2800 (4000)	So labeled; no milk
Whole wheat bread	100	None	..		9.1 (11)			910 (1100)	So labeled; no milk
Corn meal	100	Mush	+370		1.9 (2.5)	0	900	900 (1200)	
Oatmeal	100	Porridge	+300		2.0 (2.2)	0	800	800 (880)	
Rice	100	Steamed	+200	5.9	2.0	0	590	590	Cooking water absorbed
Rice Krispies	100	None	..		6.9			690	
Dairy products									
Milk	100	None	..	0.66			66	66	Raw, commercial sample
Eggs	86	Scrambled	−11	0.67	0.57	25	51	44	60–65 gm./egg.
Cheese, American	100	None	..	0.2			20	20	
Meats									
Beef									
Round	96	Fried	−25	45	56	6	4200	4100	Juices included
Liver	100	Fried	−13	120	140	0	12000	12000	Juices included
Heart	100	Steamed	−13	83	82	14	7100	7100	Cooking water used
Pork									
Bacon	100	Fried	−50	42	84	0	4200	4200	Fat discarded
Ham	84	Fried	−42	33	57	0	3300	2800	Fat discarded
Loin chops	80	Fried	−37	60	51	46	3200	2600	Juices included
Mutton									
Shoulder	75	Steamed	0	40	40	0	4000	3000	Cooking water used
Lamb leg	80	Roasted	−20	75	76	19	6100	4900	Juices included
Veal									
Chop	80	Fried	−29	71	87	13	6200	4900	Juices included
Chop	80	Steamed	0	71	65	9	6500	5200	Cooking water used
Chicken									
Leg	75	Fried	−27	38	44	15	3200	2400	Juices included
Breast	75	Fried	−7	94	100	0	9400	7100	Juices included
Fish									
Salmon	100	Fried	−13	64	69	7	6000	6000	Juices included
Salmon, canned	100	None	..		64		6400	6400	Can liquor used
Halibut	100	Steamed	0	110	104	6	11000	10000	Cooking water used
Halibut	100	Fried	−17	110	130	2	11000	11000	Juices included
Vegetables, cooked									
Asparagus, canned	100	None			7.4		740	740	Can liquor used
Beans, canned	100	None			3.5		350	350	Can liquor used
Beans, lima	100	Steamed	0	9.8 (13)[6]	9.8	0	980 (1300)[6]	980 (1300)[6]	Cooking water used
Beans, snap, canned	90	None	..		2.4		240	220	Can liquor used
Beets	55	Boiled	−7	5.6	2.8	54[5]	260	140	Tops removed. Cooked. H₂O discarded
						18	460	250	Cooked. H₂O used
Beets	55	Steamed	0	6.5	2.5	61	250	140	Cooking water used
Beet greens	100	Steamed	0	6.0	5.8	4	580	580	Cooking water used
Cabbage	80	Steamed	0	2.1	1.7	19	170	140	Cooking water used
Carrots	63	Steamed	0	3.1	3.1	0	310	200	Cooking water used
Cauliflower	60	Steamed	0	5.7	5.2	9	520	310	Cooking water used
Corn, canned	100	None	0		13		1300	1300	Can liquor used
Okra	88	Steamed	0	7.1	7.1	0	710	630	Cooking water used
Peas, canned	100	None	0		7.8		780	780	Can liquor used
Peas, dried	100	Boiled	+200	28 (35)[6]	9.3	0	2800 (3500)[6]	2800 (3500)[6]	Cooking water absorbed
Potatoes	85	Boiled	+15	14	9.3	27[5]	1000	850	Cooking water discarded
						0	1400	1200	Cooking water used
Potatoes	85	Steamed	+5	8.4	5.5	31[5]	580	490	Cooking water discarded
						0	840	710	Cooking water used
Pumpkin, canned	100	None	0		5.4		540	540	
Sauerkraut	100	Steamed	0	1.7	1.6	6	160	160	Cooking water used
Spinach	83	Steamed	0	5.1	4.3	16	430	360	Cooking water used
Tomatoes, canned	100	None	0		4.7		470	470	Can liquor used
Turnips	66	Boiled	+3	9.7	5.6	40[5]	580	380	Cooking water discarded
						0	970	640	Cooking water used
Vegetables, raw									
Cabbage	80			2.1			210	170	
Carrots	63			3.1			310	200	
Lettuce	95			2.5			250	240	
Tomatoes	97			4.7			470	460	
Fruits, cooked									
Pears, canned	100	None	0		1.7		170	170	Can liquor used
Prunes, canned	97	None	0		2.4		240	230	Can liquor used
Fruits, raw									
Apples	91			0.81			81	74	
Bananas	68			5.8			580	400	
Canteloup	50			10			1000	500	
Grapefruit	66			2.1			210	140	
Oranges	63			3.0			300	190	
Peaches, frozen	100			3.3			330	330	
Raisins	100			2.9			290	290	
Strawberries	97			2.2			220	210	
Watermelon	59			2.4			240	140	
Miscellaneous									
Peanuts	69			86			8600	5900	
Chocolate	100			11			1100	1100	
Molasses	100			39			3900	3900	

[1] See footnotes 1, 2, 3 4 and 5 to table 1
[6] Nicotinic acid values in parentheses represent assays of acid hydrolyzed extracts

TABLE 3
Yield of pantothenic acid per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	PANTOTHENIC ACID Raw edible portion	Cooked edible portion	Total loss [2]	Yield of pantothenic acid in micrograms Per 100 gm. raw edible portion [4]	Per 100 gm. as purchased [4]	REMARKS
	%[1]		%E.P.[2]	µg/gm.	µg./gm.	%			
Cereal products									
White bread	100	None	..		4.6			460	Approx. 2% milk solids
Rye bread	100	None	..		5.1			510	So labeled; no milk
Whole wheat bread (100%)	100	None	..		5.7			570	So labeled; no milk
Whole wheat bread	100	None	..		4.2			420	So labeled; no milk
Corn meal	100	Mush	+370		0.66	0	310	310	Cooking water absorbed
Oatmeal	100	Porridge	+300		3.2	0	1300	1300	Cooking water absorbed
Rice	100	Steamed	+200	3.5	1.2	0	350	350	Cooking water absorbed
Rice Krispies	100	None			3.4			340	
Dairy products									
Milk	100	None		2.9			290		Raw, commercial sample
Eggs	86	Poached	0	14	14	0	1400	1200	60–65 gm./egg
Cheese, American	·100	None	..	2.3			230	230	
Meats									
Beef									
Round	96	Fried	−25	4.9	4.4	32	330	250	Juices included
Liver	100	Fried	−13	76	75	14	6500	6500	Juices included
Heart	100	Steamed	−13	20	14	37	1300	1300	Cooking water used
Pork									
Bacon	100	Fried	−50	5.2	9.2	12	460	460	Fat discarded
Ham	84	Fried	−42	5.0	8.6	0	500	320	Fat discarded
Loin chops	80	Fried	−37	4.4	4.8	31	300	240	Juices included
Mutton									
Shoulder	75	Steamed	0	4.3	4.3	0	430	320	Cooking water used
Lamb leg	80	Roasted	−20	6.0	6.8	12	530	420	Juices included
Veal									
Chop	80	Fried	−29	1.9	2.3	14	160	130	Juices included
Chicken									
Leg	75	Fried	−27	6.2	6.1	28	450	340	Juices included
Breast	75	Fried	−7	5.3	4.9	14	460	350	Juices included
Fish									
Salmon	100	Fried	−13	6.6	7.6	0	660	660	Juices included
Salmon, canned	100	None	..		4.5		450	450	Can liquor used
Halibut	100	Steamed	0	1.5	1.5	0	150	150	Cooking water used
Halibut	100	Fried	−17	1.5	1.8	0	150	150	Juices included
Vegetables, cooked									
Asparagus, canned	100	None	0		1.4		140	140	Can liquor used
Beans, canned	100	None	0		1.8		180	180	Can liquor used
Beans, lima	100	Steamed	0	8.3	6.1	26	610	610	Cooking water used
Beans, snap, canned	90	None	0		1.1		110	99	Can liquor used
Beets	55	Boiled	−7	1.3	1.1	24[5]	99	54	Tops removed. Cooked. H₂O discarded
						18	107	59	Cooked. H₂O used
Beets	55	Steamed	0	1.1	0.68	38	68	38	Cooking water used
Beet greens	100	Steamed	0	1.4	0.96	36	96	96	Cooking water used
Cabbage	80	Steamed	0	1.8	1.3	28	130	110	Cooking water used
Carrots	63	Steamed	0	2.5	2.3	10	230	140	Cooking water used
Cauliflower	60	Steamed	0	9.2	8.9	3	890	540	Cooking water used
Corn, canned	100	None	0		3.2		320	320	Can liquor used
Okra	88	Steamed	0	2.1	2.1	0	210	180	Cooking water used
Peas, canned	100	None	0		1.3		130	130	Can liquor used
Peas, dried	100	Boiled	+200		4.3		1300	1300	Cooking water absorbed
Potatoes	85	Boiled	+5	3.2	1.9	40[5]	190	160	Cooking water discarded
						0	320	270	Cooking water used
Pumpkin, canned	100	None	0		1.3		130	130	
Sauerkraut	100	Steamed	0	0.57	0.57	0	57	57	Cooking water used
Spinach	83	Steamed	0	1.8	1.7	6	170	140	Cooking water used
Tomatoes, canned	100	None	0		4.1		410	410	Can liquor used
Turnips	66	Boiled	+3	1.4	0.8	41[5]	83	55	Cooking water discarded
						8	130	86	Cooking water used
Vegetables, raw									
Cabbage	80			1.8			180	140	
Carrots	63			2.5			250	160	
Lettuce	95			1.1			110	100	
Tomatoes	97			3.7			370	360	
Fruits, cooked									
Pears, canned	100	None			0.20		20	20	Can liquor used
Prunes, canned	97	None			0.43		43	42	Can liquor used
Fruits, raw									
Apples	91			0.60			60	55	
Bananas	68			1.8			180	120	
Canteloup	50			2.3			230	115	
Grapefruit	66			2.9			290	190	
Oranges	63			3.4			340	210	
Peaches, frozen	100			1.7			170	170	
Raisins	100			0.90			90	90	
Strawberries	97			2.6			260	250	
Watermelon	59			3.1			310	180	
Miscellaneous									
Peanuts	69			25			2500	1700	
Chocolate	100			1.9			190	190	
Molasses	100			2.6			260	260	

See footnotes 1, 2, 3, 4 and 5 to table 1.

TABLE 4

Riboflavin, nicotinic acid and pantothenic acid contributions from various foods to make a total of 2500 calories.

FOODS	GM	CAL.	CAL. % OF TOTAL	RIBOFLAVIN µg	RIBOFLAVIN % of total	NICOTINIC ACID µg	NICOTINIC ACID % of total	PANTOTHENIC ACID µg	PANTOTHENIC ACID % of total
White bread (includes some sugar and fat)	260 [1]	679	27.3	240	16.0	1600 (1800) [2]	13.9 (15.4) [2]	1200	26.2
Rye bread	4	9	0.4	3	0.2	37 (44) [2]	0.3 (0.4) [2]	20	0.4
Whole wheat bread	6	16	0.6	4	0.3	55	0.5	25	0.5
Corn meal	11	41	1.6	7	0.5	99 (120) [2]	0.9 (1.0) [2]	34	0.7
Oatmeal	4	17	0.7	4	0.3	32 (35) [2]	0.3	52	1.1
Prepared breakfast cereals	6	20	0.8	3	0.2	41	0.4	17	0.4
Rice	5	16	0.6	3	0.2	30	0.3	18	0.4
Sugar (apart from bread)	97	384	15.3	0	0	0	0	0	0
Total cereal products	393	1182	47.3	260	18	1900 (2100) [2]	17 (18) [2]	1360	30
Milk	320	222	8.9	540	37.3	210	1.8	930	20.3
Eggs	34	48	1.9	120	8.3	15	0.1	410	9.0
Cheese	8	30	1.2	47	3.2	1.6	0	18	0.4
Butter	20	148	5.9	2	0.1	0	0	0	0
Total dairy products	107	448	17.9	710	49	230	1.9	1360	30
Bacon (including fat pork)	7	43	1.7	19	1.3	300	2.7	32	0.7
Pork (other than bacon)	39	155	6.2	62	4.3	1100	9.8	130	2.8
Mutton and lamb	6	16	0.6	13	0.8	240	2.2	22	0.5
Beef	46	104	4.2	74	5.1	1900	16.9	140	3.1
Veal	7	10	0.4	9	0.6	360	3.2	11	0.2
Poultry	15	21	0.8	21	1.5	750	6.7	52	1.1
Fish	20	14	0.6	20	1.4	1600	14.2	70	1.5
Liver and other organs	3	3	0.1	60	4.1	300	2.7	150	3.3
Total flesh	143	366	14.6	280	19	6600	59	610	13
Fats and oils [3]	19	171	6.8	0	0	0	0	0	0
Asparagus, canned [4]	4	1	..	4	0.3	30	0.3	6	0.1
Beans, baked, canned [4]	7	7	0.3	3	0.2	25	0.2	13	0.3
Beans, lima, canned [4]	2	2	..	3	0.2	20	0.2	130	2.8
Beans, snap	11	4	0.1	5	0.3	24	0.2	11	0.2
Beets	4	1	..	1	0.1	10	0.1	2	0
Cabbage, boiled (inc. cauliflower)	15	3	0.1	5	0.3	35	0.3	50	1.1
Cabbage, raw [5]	5	1	..	2	0.1	10	0.1	7	0.1
Carrots, boiled	10	3	0.1	4 (est.)	0.3	20	0.2	14	0.3
Carrots, raw [5]	3	1	..	1	0.1	9.3	0.1	5	0.1
Corn, canned [4]	13	13	0.5	10	0.7	170	1.5	42	0.9
Okra (including eggplant)	4	2	..	3	0.2	25	0.2	7	0.1
Onions	21	9	0.4	4	0.3	15 (est.)	0.1	25 (est.)	0.5
Lettuce	12	1	..	3	0.2	30	0.3	12	0.3
Parsnips and radishes	2	1	..	1	0.1				
Peas, canned [4]	13	7	0.3	13	0.9	100	0.9	17	0.4
Peas and beans, dried	7	24	1.0	11	0.8	140 (170) [2]	1.2 (1.5) [2]	65	1.4
Potatoes	138	100	4.0	41	2.9	950	8.4	220	4.8
Pumpkin, canned [4]	7	3	0.1	4	0.3	38	0.3	9	0.2
Sauerkraut	3	1	..	1	0.1	4.8	0	2	0
Spinach	6	1	..	8	0.6	22	0.2	8	0.2
Sweet potato	20	22	0.9	7	0.5	100 (est.)	0.9	180 (est.)	3.9
Tomatoes, canned [4]	18	4	0.2	5	0.3	85	0.7	74	1.6
Tomatoes, raw [5]	18	4	0.2	5	0.3	85	0.7	67	1.5
Turnips	4	1	..	1 (est.)	0.1	15	0.1	2	0
Total vegetables	347	219	8.8	145	10	2000	17	970	21
Apples, cooked	19	11	0.5	2	0.1	15 (est.)	0.1	12 (est.)	0.3
Apples, (raw, including pears) [6]	37	21	0.8	6	0.4	37	0.3	20	0.4
Bananas	25	16	0.6	10	0.7	100	0.9	30	0.7
Strawberries (including other berries)	5	2	..	2	0.1	11	0.1	13	0.3
Canteloup	7	1	..	1	0.1	35	0.3	8	0.2
Grapes	5	3	0.1						
Oranges and other citrus fruits	51	18	0.7	13	0.9	90	0.8	100	2.2
Peaches	10	5	0.2	2	0.1	33	0.3	17	0.2
Prunes, cooked	5	11	0.4	2	0.1	12	0.1	2	0
Raisins	2	6	0.2	0	0	6	0.1	2	0
Watermelon	15	2	0.1	5	0.3	21	0.2	27	0.6
Total fruits	181	96	3.9	43	3.0	360	3.2	230	4.9
Peanuts (including other nuts)	2	12	0.5	2	0.1	120	1.1	34	0.7
Chocolate	1	8	0.3	2	0.1	11	0.1	2	0
Total miscellaneous	3	20	0.8	4	0.2	130	1.2	36	0.7
Grand total	1193	2502	100.2	1440	99	11200 (11400) [2]	99	4600	99

[1] Sufficient white bread was included in the diet to account for the total average white flour consumption. Part of this, of course, appears as other bakery goods, macaroni, etc., in customary human diets.

[2] Nicotinic acid values in parentheses represent assays of acid hydrolyzed extracts.

[3] This does not include fats contained in meats. A portion of the total shortening appears in the bread, that amount being deducted from the average consumption of fats and oils.

[4] For convenience we used and analyzed canned product but it should not be inferred that the product is not used in customary diets in other ways.

[5] All foods other than fruits were cooked except when otherwise indicated.

TABLE 5

The riboflavin content of foods of different variety and source.

MATERIAL	SOURCE	BRAND OR VARIETY	NUMBER OR QUANTITY OF SPECIMENS TESTED	DRY WEIGHT %	RIBOFLAVIN CONTENT EDIBLE PORTION Fresh μg./gm.	RIBOFLAVIN CONTENT EDIBLE PORTION Dry μg./gm.
Apples	Washington	Delicious	9	15.0	0.15	1.00
	Washington	Delicious	6	14.0	0.13	0.93
	Washington	Rome Beauty	9	14.0	0.16	1.14
	New York	Greening	10	14.4	0.21	1.46
	New York	MacIntosh	10	13.8	0.17	1.23
	New Jersey	Baldwin	8	14.5	0.20	1.37
Asparagus, canned	California	Del Monte	16 oz.	6.4	0.62	9.7
	California	Del Monte	14 oz.	6.4	0.54	8.4
	?	Rob-Ford	18 oz.	6.0	1.07	18
Carrots	Texas	?	7	13.6	0.43	3.2
	California	?	15	13.5	0.50	3.7
Cheese	Wisconsin	Cheddar	3	74.0	6.2	8.4
	Wisconsin	Mild cream	1	65.0	5.4	8.3
	Wisconsin	Proc. cheddar	1	62.0	5.1	8.2
Eggs	Oregon	White Leghorn	12	25.8	2.2	8.5
	Oregon	White Leghorn	12	26.0	4.2	16
	Oregon	R. I. Red	12	26.0	4.5	18
	Oregon	R. I. Red	12	26.0	5.1	20
	New Jersey	White Leghorn	12	26.8	6.4	24
	New Jersey	White Leghorn	12	26.5	3.6	14
	New Jersey	R. I. Red	12	26.0	5.2	20
	Texas	White Leghorn	12	25.5	4.2	17
Lettuce	Arizona	Head	1	5.2	0.33	6.3
	California	Head	1	5.0	0.20	4.0
Onions	Texas	White	10	10.6	0.23	2.2
	Texas	Brown	4	12.5	0.24	1.9
Oranges	Florida	?	6	13.0	0.51	3.9
	California	Navel	6	16.0	0.42	2.6
Parsnips	Pennsylvania	?	6	24.4	0.63	2.6
	Texas	?	9	22.8	0.91	4.0
Peanuts	Brazil	?	70 gm.	98.4	0.98	1.00
	Virginia	?	70 gm.	98.0	1.12	1.14
Potatoes	Maine	Green Mtn.	6	16.6	0.36	2.2
	Idaho	Netted Gem	6	20.0	0.23	1.2
	Florida	Early Rose (new)	9	11.4	0.29	2.5
Raisins	California	Seedless	100 gm.	76	0.29	0.38
	California	Seeded	100 gm.	75	0.20	0.27
Sweet potatoes	S. Carolina	Yams	7	29.2	0.40	1.4
	New Jersey	White	7	36.2	0.45	1.2
Tomatoes	California	?	4	5.2	0.24	4.6
	California	?	4	6.8	0.24	3.5

about one-seventh, and potatoes with one-twelfth of the total, are the only other important contributors.

The present value of 11 mg. daily average intake of nicotinic acid rates as marginal or low when compared with the estimated requirements of this vitamin (Elvehjem, '41; Williams, '42). Enrichment of bread, on the other hand, has provided substantial increases in the average intake. The prevailing addendum of 10 mg. of nicotinic acid per pound of bread results in a total intake of approximately 17 mg. per 2500 calories.

Pantothenic acid. This vitamin, like thiamine, is more evenly distributed than riboflavin or nicotinic acid among the various classes of foods comprising the average American diet. Cereals, dairy products and fresh vegetables each furnish about one-fourth of the total supply of pantothenic acid. The individual important sources are white bread, milk, eggs and potatoes, in the order named.

Data regarding the nutritional significance and requirements of pantothenic acid are meager. The estimated daily requirements of 9 to 11 mg. by Gordon ('42) and of 11 mg. by Williams ('42) are far in excess of the actual average intake. Deficiencies of this vitamin, if they exist, do not appear to be due in large measure to milling losses during flour manufacture. Restoration by enrichment of bread with pantothenic acid would therefore result in only a slight increase in the daily supply.

CONCLUSIONS

The riboflavin, nicotinic acid and pantothenic acid contents of the average American diet, such as was consumed by the middle two-thirds or three-fourths of the population prior to the use of enriched bread and flour, are approximately 1.4 mg., 11 mg., and 4.9 mg., respectively, per 2500 calories. These values are appppreciably lower than the most generally supposed daily requirements.

Enrichment of bread and flour to meet prevailing standards increases the level of riboflavin, 12% to 1.6 mg.; that of nicotinic acid, 53% to 17 mg.

The principal contributors of these vitamins to ordinary diets are as follows in the order of their importance: for riboflavin, milk, white bread, eggs, potatoes and liver; for nicotinic acid, beef, lean pork, fish, white bread and potatoes; for pantothenic acid, white bread, milk, eggs and potatoes.

Tables are presented to permit the calculation of vitamin contents of other diets.

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THE MINERAL COMPOSITION OF THE ALBINO RAT AS AFFECTED BY CHLORIDE DEFICIENCY [1]

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In a previous report (Voris and Thacker, '42) it was shown that the substitution of bicarbonate for chloride in the diets of rats served to diminish their growth, appetite, and gain of nitrogen and energy, and to increase their water consumption and heat production. Thus, the nutritional effects of a deficiency of dietary chloride are, in many respects, similar to those of deficiencies of sodium and potassium (Orent-Keiles, Robinson and McCollum, '37; Kahlenberg, Black and Forbes, '37; Orent-Keiles and McCollum, '41).

Recently, Greenberg and Cuthbertson ('42) found that rats fed a low chloride diet gained less in weight, and had lower blood chloride and higher total carbon dioxide contents than did rats which received a diet of normal chloride content. Since no significant difference was found between the pH values of the whole blood of the chloride deficient and the control animals, the alkalosis arising from the increased carbon dioxide content of the blood was, then, virtually compensated.

However, though the chloride ion is one of the chief constituents of the body fluids, little is known of the specific rôle of chloride in its relations to other mineral elements. In the present investigation, the metabolism of chloride, sodium, potassium, calcium, magnesium, and phosphorus in rats as affected by feeding a diet deficient in chloride has been studied.

EXPERIMENTAL

This study was carried out in conjunction with an experiment previously reported from this laboratory (Voris and Thacker, '42). The composition of the diets and details of experimental procedure were the same in both investigations and will not be repeated here.

The bodies, feces and urines of all the rats were analyzed for chloride, sodium, potassium, calcium, and magnesium, except that only chloride was determined on the urine of the chloride deficient rats on account of the loss of samples during the ashing process. Composite samples of the male and female bodies were analyzed for phosphorus.

The analytical methods used were as follows: chloride, Sendroy's titrimetric iodate method (Sendroy, '37); sodium, Barber and Kolthoff ('28, '29); potassium, the chloroplatinate separation with the potassium estimated by reducing the potassium chloroplatinate with powdered metallic magnesium, then determining the released chloride ion by Sendroy's titrimetric iodate method; calcium and magnesium, as outlined by Hillebrand and Lundell ('29); phosphorus, on the recombined rat bodies (ether extract recombined with the body residues) after wet digestion, by the volumetric method of the Association of Official Agricultural Chemists ('30).

Sodium, potassium, calcium, and magnesium were determined on aliquots of the diets, body residues, feces, and urines ashed overnight at a dull red heat (550° C.). Chloride was determined on the fresh urines, and on 2.5 gm. samples of the diets, body residues, and feces containing 5 ml. of a 20% solution of sodium carbonate, and ashed overnight at a dull red heat (550° C.).

Twelve rats (six male, six female), corresponding in age, weight and breeding to the paired rats used as experimental subjects, were killed and analyzed to represent the mineral composition of the experimental rats at the start of the feeding experiment.

The gain in each mineral during the experimental period, therefore, was determined by the difference between the com-

position of the rats at the end of the experiment and of the controls selected to represent these animals at the beginning of the experimental feeding. The average analyses for males and females were used separately.

RESULTS AND DISCUSSION

Table 1 summarizes the composition of the rations with respect to chloride, sodium, potassium, calcium, magnesium, nitrogen, energy and dry matter. Although the diets were not analyzed for phosphorus, the approximate phosphorus content was 0.153%. It was intended to have both the deficient and the control rations of similar mineral composition, except for chloride content. This objective was accomplished reasonably well except with reference to sodium.

Inasmuch as the growth data have already been adequately discussed by Voris and Thacker ('42), the data in table 2 are presented primarily to indicate the derivation of the calculated values in the following tables.

The average percentage composition of the seven male and five female rats of the normal and of the chloride deficient groups as to chloride, sodium, potassium, calcium, magnesium, and phosphorus is given in table 3. These values are also expressed as percentages of the fat-free tissue, and as millimoles per kilogram of body water. In addition, the retention of these minerals, computed by comparison of the composition of the bodies of the chloride deficient rats at the end with that representing their composition at the beginning of the experimental feeding, is recorded as total milligrams, as per cent of fat-free tissue gained, and as millimoles per kilogram of water gained.

Inspection of the standard errors reveals that when the mineral composition is expressed as a function of body weight or water content, the individual variation is surprisingly small, though the final live weight of the rats varied as much as 62 gm. Mineral composition or mineral retention has little significance unless correlated with body weight or with water content.

TABLE 1

Composition of rations.

RATION	NITROGEN	ENERGY	DRY MATTER	CHLORIDE	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM
	%	Cals/gm	%	%	%	%	%	%
Normal chloride[1]	2.89	4.66	98.6	0.28	0.102	0.315	0.617	0.0381
Chloride deficient	2.89	4.66	98.6	0.02	0.120	0.305	0.624	0.0366

[1] Average of two batches.

TABLE 2

Growth data of normal control and chloride deficient rats for a 70-day period. Averages for seven males and five females.

	LIVE WEIGHT[1]	FAT-FREE TISSUE[1]	GAIN OF BODY TISSUE	GAIN OF FAT-FREE TISSUE	BODY WATER	GAIN OF WATER
	gm.	gm.	gm.	gm.	gm.	gm.
♂ Control	176.7	158.7	113.0	100.7	113.8	70.3
♂ Chloride deficient	147.6	134.9	83.7	76.6	95.8	52.1
♀ Control	158.5	137.8	104.5	89.1	98.8	61.5
♀ Chloride deficient	129.1	114.4	75.8	66.4	81.1	44.7

[1] Does not include the contents of alimentary tract.

TABLE 3

Mineral constituents of the bodies of normal control and of chloride deficient rats. Averages for seven males and five females.[1]

	PER CENT OF LIVE WEIGHT[2]	PER CENT OF FAT FREE TISSUE	MILLIMOLES PER KILOGRAM BODY WATER	RETENTION DURING 70 DAYS Total (mg.)	RETENTION DURING 70 DAYS Per cent of fat-free tissue gained	RETENTION DURING 70 DAYS Millimoles per kilogram water gained
Chloride						
♂ Control	0.098 ± .002	0.109 ± .002	43.0 ± .65	102.2	0.101 ± .003	41.0 ± 1.05
♂ Chloride deficient	0.087 ± .002	0.096 ± .002	37.9 ± .62	57.5	0.075 ± .003	31.2 ± 1.15
♀ Control	0.092 ± .002	0.106 ± .003	41.7 ± .96	84.4	0.095 ± .003	38.8 ± 1.53
♀ Chloride deficient	0.085 ± .001	0.096 ± .001	38.3 ± .53	50.1	0.075 ± .002	31.5 ± .78
Sodium						
♂ Control	0.107 ± .002	0.119 ± .002	72.2 ± 1.13	118.2	0.117 ± .003	73.1 ± 1.85
♂ Chloride deficient	0.105 ± .0005	0.115 ± .0006	70.5 ± .40	84.5	0.110 ± .001	70.5 ± .77
♀ Control	0.107 ± .0008	0.124 ± .004	75.0 ± 2.27	111.3	0.126 ± .006	79.8 ± 3.96
♀ Chloride deficient	0.103 ± .002	0.116 ± .001	71.0 ± .56	74.7	0.113 ± .002	72.6 ± 1.20
Potassium						
♂ Control	0.268 ± .002	0.298 ± .003	106.4 ± 1.03	303.9	0.301 ± .005	110.4 ± 1.50
♂ Chloride deficient	0.261 ± .002	0.286 ± .002	103.0 ± .78	215.5	0.280 ± .004	105.6 ± 1.47
♀ Control	0.264 ± .004	0.303 ± .004	108.6 ± 1.54	273.5	0.308 ± .007	118.4 ± 6.38
♀ Chloride deficient	0.261 ± .002	0.294 ± .002	106.0 ± .50	194.3	0.293 ± .003	111.1 ± 1.07
Calcium						
♂ Control	1.20 ± .03	1.34 ± .03		1535	1.53 ± .05	
♂ Chloride deficient	1.24 ± .02	1.36 ± .02		1239	1.61 ± .04	
♀ Control	1.21 ± .04	1.40 ± .04		1418	1.62 ± .08	
♀ Chloride deficient	1.33 ± .04	1.49 ± .03		1218	1.84 ± .07	
Magnesium						
♂ Control	0.050 ± .001	0.055 ± .001	31.8 ± 0.69	59.1	0.059 ± .002	34.6 ± 1.14
♂ Chloride deficient	0.048 ± .001	0.053 ± .001	30.4 ± .76	41.9	0.055 ± .002	33.1 ± 1.42
♀ Control	0.048 ± .0006	0.055 ± .0006	31.8 ± 1.07	52.8	0.060 ± .001	39.7 ± 4.71
♀ Chloride deficient	0.050 ± .0005	0.057 ± .0001	32.9 ± .16	42.0	0.063 ± .001	38.1 ± .56
Phosphorus						
♂ Control	0.636	0.708		773.4	0.768	
♂ Chloride deficient	0.696	0.761		675.0	0.881	
♀ Control	0.589	0.677		675.5	0.758	
♀ Chloride deficient	0.705	0.795		655.6	0.987	

[1] Plus and minus values are the standard errors of the means.
[2] Contents of alimentary tract removed.

Since the individual results were paired variates, the significance of any differences between the control and the chloride deficient rats was determined by Fischer's ('28) modification of "Student's" table. Since little or no difference in mineral composition was found between the sexes, both male and female rats were combined in the comparisons between the chloride and the bicarbonate animals.

By expressing mineral constituents as percentages of the fat-free tissue, the highly variable fat gain was segregated, thereby increasing the statistical significance of the data. Therefore, for the sake of brevity, only the results expressed as per cent of fat-free tissue will be discussed.

There were no significant differences in mineral composition between the male and female rats of the control group, but the chloride deficient animals did show small significant differences between the sexes (as determined by the standard error of the difference). The potassium, calcium, magnesium, and phosphorus constituents of the fat-free bodies were significantly higher in the females than in the males, the differences being respectively, 2.5, 2.5, and 4 times their standard errors.

The chloride content of the chloride deficient rats was 88.9% (value for "P" was less than .01) of that of the control animals. This difference was surprising only in that it was no larger, since the chloride deficient ration contained only 7% as much chloride as did the normal ration. The rat accomplished this adaptation by a slower rate of growth, and by conservation of the available chloride.

When the sodium and potassium composition of the fat-free tissue of the chloride deficient and of the control groups was compared, the composition of the deficient animals in these mineral elements was 95.0 and 96.7% ("P" value less than .01 in both cases), respectively, of that of the controls.

The phosphorus contents of the fat-free bodies of the males and females of the chloride deficient group were 107.5 and 117.4%, respectively, of those of the male and female animals of the normal chloride group. Though statistical tests of significance are lacking for these differences, it would not be fal-

lacious, considering the consistent character of the previous data, to assume that the differences were significant and due to an insufficiency of chloride in the diet.

No real differences were evident between the two groups as to the calcium and magnesium contents of the rat bodies.

The relation of mineral retention to gain of body tissue reveals that the contents of the fat-free tissue gain of the chloride deficient rats in chloride, sodium, and potassium were 72.7, 91.7, and 94.1% ("P" value of less than .01 in all cases), respectively, of the contents of the control animals in these elements.

However, the calcium content of the fat-free gain of the chloride deficient rats was 109.6% ("P" value less than .01) of that of the control animals. This difference signifies that the rats receiving the chloride deficient ration made a greater skeletal growth in proportion to the tissue growth than did the rats receiving the ration of normal chloride content. The phosphorus content of the body gain lends support to this interpretation since the chloride deficient animals gained 122.4% as much phosphorus as did the control rats.

The magnesium content of the gains did not vary significantly between the two groups.

Of the minerals determined, chloride, sodium, potassium, and to some extent, magnesium are of importance as electrolytes; therefore, their relation to body water is significant in relation to the effects of dietary chloride deficiency.

In table 3 the millimole retention of these minerals is related to the kilograms of water gained. Again, the results show a relatively slight variation within the group, and the individual differences are highly consistent.

A comparison of the two groups shows that the concentrations of chloride, sodium, and potassium in the water gain were 78.1, 94.2, and 96.3% ("P" value less than .02 in all cases), respectively, of the concentration of these elements in the water gain of the control animals. The magnesium concentration was not significantly affected by the chloride deficiency.

The chloride deficient rats, to compensate for the lower concentration of chloride, gained a lower percentage of water than did the normal chloride rats (Voris and Thacker, '42), and also reduced the concentration of sodium and potassium in that water.

In addition to the values for mineral retention computed from the body composition, it was planned to determine retention from the analysis of feeds and excreta, but this objective was accomplished only in part, as shown in table 4 in which complete balances are given for sodium, potassium, calcium, magnesium and chloride in the control rats; the balances for the chloride deficient rats being complete with reference to chloride only, and incomplete for the other elements studied.

Though the feces of the deficient rats contained approximately one-half as much chloride as did the feces of the control animals, their chloride retention was 50.3 to 51.3% of that in the feed; whereas, the control rats retained 5.4 to 6.7%. The ability of rats to conserve chloride when their supply is severely limited was illustrated by the urinary excretion of this element. The urinary chloride excretion of the chloride deficient rats was approximately 40% of their intake, or less than 0.6 mg. daily; while the control animals excreted in the urine over 90% of their intake, or nearly 200 mg. daily.

Based on the results of the body analysis of the rats, with both the males and the females the percentage retention of the intake of sodium, potassium, calcium and magnesium was lower for the chloride deficient rats than for the normal controls.

The agreement between the retentions of chloride, potassium, calcium, and magnesium obtained by the two analytically unrelated methods (tables 3 and 4) attests to the accuracy of the analytical work.

The retention of sodium, however, as determined by the two methods, was at variance. The retention calculated from the excreta analysis was, on the average, 38.1 ± 1.52 [2] and 35.9 ± 4.86 [2] % higher for the male and female rats, respec-

[2] Standard error.

TABLE 4

Mineral constituents of the feed, feces, and urine of normal control and of chloride deficient rats. Averages for seven males and five females.

	CHLORIDE	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM
	mg.	*mg.*	*mg*	*mg.*	*mg.*
♂ Control					
Intake	1504.5	537.9	1710.9	3384	199.1
Feces	21.3	26.0	70.6	1720	51.4
Urine	1385.3	320.8	1362.7	136	87.3
Retention	100.7	191.1	277.7	1528	61.8
♂ Chloride deficient					
Intake	106.3	637.7	1620.9	3316	194.5
Feces	10.5	14.5	38.0	1973	41.6
Urine	42.3				
Retention	53.5				
♀ Control					
Intake	1439.5	514.8	1636.3	3238	190.6
Feces	18.7	25.9	65.1	1625	51.5
Urine	1341.3	315.4	1283.6	156	79.4
Retention	77.3	173.5	287.7	1457	59.7
♀ Chloride deficient					
Intake	101.6	609.6	1549.5	3170	185.9
Feces	10.3	15.7	41.5	1862	39.3
Urine	39.2				
Retention	52.1				

tively, than the values obtained by body analysis. The significance of this difference was not determined.

In harmony with the well-known reciprocal relationship between chloride and bicarbonate ions (Peters and Van Slyke, '31; Greenberg and Cuthbertson, '42) was the finding in the present investigation that the decrease of 8.8 mM in the chloride in the water gain was accompanied by a decrease of 4.4 mM in the concentration of sodium.

Since sodium is present, for the most part, in the extracellular fluids, and potassium is present largely in the intracellular fluids, a decrease in the concentration of sodium would necessitate a similar decrease in the potassium in order to maintain the osmotic equilibrium of the two fluids. The decrease of 4.1 mM in the potassium concentration of the water gain indicated, as did the Na/K ratio, that the diminished sodium concentration was counterbalanced by a similar decrease in the potassium concentration.

SUMMARY

In a 70-day paired feeding experiment comparative data on the metabolism of chloride, sodium, potassium, calcium, magnesium and phosphorus were secured by body analysis of rats on a synthetic diet of normal chloride content (0.28%) and animals on a similar diet in which the chlorides of the salt mixture were replaced by equivalent quantities of corresponding bicarbonates. The chloride deficient ration contained 0.02% chloride and 0.49% bicarbonate. The bicarbonate being considered innocuous, the results obtained were attributed to the deficiency of dietary chloride.

In comparison with the rats receiving the normal chloride ration, the contents of the bodies of the rats receiving the chloride deficient ration were less in chloride, sodium, and potassium and greater in calcium and phosphorus; the contents of the body gain were less in chloride, sodium, and potassium, and greater in calcium and phosphorus; and the concentration of chloride, sodium and potassium was less in the total water and water gain without disturbance of the ratio of

sodium to potassium. The adjustments of the female rats to a restricted intake of chloride were similar to but not as large as those of the male rats.

The percentage retention of the intake of sodium, potassium, calcium and magnesium was lower for the chloride deficient rats than for the normal controls; while the percentage retention of chloride was much higher for the chloride deficient rats than for the controls.

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A STUDY OF THE DIET AND NUTRITIONAL STATUS OF WOMEN IN A LOW-INCOME POPULATION GROUP [1]

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The variation of vitamin and mineral content of food materials with such factors as source and season and the recognized but not readily assessed losses during storage, preservation, and preparation make a study of the nutritive value of diets as they are actually consumed of considerable interest. In this investigation a study was made of the diets of twenty-four women from the low-income group, all of whom lived, during the time they participated, in the various projects of the Austin Housing Authority. Anglo-Americans, Latin-Americans, and Negroes were represented. Diets were collected during one, two, or three weekly periods from November to June and examined quantitatively with respect to thiamine, riboflavin, niacin, pantothenic acid, protein, calcium, phosphorus, and calories. The analytical findings were correlated with data obtained by physical examination of fifteen of the subjects for anatomical evidences of deficiency.

EXPERIMENTAL

Selection of subjects

The subjects selected for study were residents of the three housing projects located in Austin, Texas and included Anglo-Americans, Latin-Americans, and Negroes. The mothers in

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the families, chosen on the recommendation of the project director, were asked to participate because it was thought that they would be likely to be at home for all meals, would be interested, and would be more careful than any other member of the family in collecting the food samples. In order to have as homogeneous a group as possible, care was taken to select families of like size and income. The diets of five women from each racial group were collected for a period of 1 week at three different times during the year so that seasonal variation might be studied. From only eight of the women who cooperated in the study was it possible to obtain collections for all three periods as some of the families moved from the project, others had illnesses in the family, and some of the women decided that collecting the sample was too troublesome. Four women in both the Latin-American and Negro groups made collections for the three periods, but none of the Anglo-American women made collections for more than 2 weeks. The husbands in the families studied were occupied as plumber's helpers, day laborers, truck drivers, porters, etc. The weekly incomes of the twenty-four families ranged from ten to eighteen dollars and averaged thirteen dollars and fifty cents.

Collection of samples

The samples collected daily were, as nearly as possible, duplicates of the day's diet of the women participating, and included all meals and between meal snacks. Every effort was made to impress the women with the necessity of care in making their collections. They were instructed to have an extra plate on the table at each meal and to put on this plate exactly the same amount of food as they ate. At the end of each meal the food on the extra plate was to be transferred to a ½-gallon Mason jar, — milk, soup or other liquids consumed, except water, coffee, and tea, were to be put in a separate jar. The jars were furnished for this purpose. Corresponding portions of everything eaten between meals were also to be added to the jars. The women were asked to make lists of what they ate each day, so that the contents of the jar might be qualita-

tively checked. The women were reimbursed at the end of each weekly period in the amount of 25 cents per day.

The reliability of collections made in the way described evidently depends on the reliability of the subjects. In this study the cooperative spirit and interest of the women participating and the careful check made at the time of collection seemed to insure that the samples collected represented intake with reasonable accuracy. The probable reliability of the samples is discussed in more detail later on.

Preparation of samples

The solid food material was first ground in a food chopper. The liquid portion, together with water used in washing the grinder, was added and the mixture homogenized in a Waring Blendor. The homogeneous suspension was then made to a volume of 2 liters. For the dry weight determination a 20-ml. aliquot was dried in an electric oven at 70° C. for 24 hours. For thiamine assay, a 15 ml. aliquot was acidified with dilute H_2SO_4 (using Congo red paper) and the acidified solution steamed for 20 minutes. A portion of this acid extract was sulfite treated following the directions of Frey, Atkin, and Schultz ('42). For riboflavin, pantothenic acid, and niacin assay a 10-ml. aliquot was mixed with 30 ml. sodium acetate-acetic acid (0.5%) buffer, pH 4.5, 20 mg. each of clarase and papain added (approximately 2% by weight), and the solution placed in a 37° C. incubator for 24 hours. The sample was then steamed 30 minutes, cooled, restored to volume, centrifuged, tubed, and steamed 10 minutes for sterilization. Aliquots of this extract were used for the microbiological assays.

For the mineral assays 60 ml. of each of the seven samples for a period were combined, and a 25-ml. aliquot of this total sample digested by the perchloric acid method of Gerritz ('35) and eventually evaporated to between 20 and 50 ml. Complete hydrolysis was insured by the addition of 20 ml. water and further heating. The solution was then made to 250 ml. Aliquots of this 250-ml. solution were used for the mineral determinations.

After the sample necessary for the mineral analyses had been taken from the mixed week's sample, as described under preparation of sample for mineral assays, the remainder of the material was dried and used for protein assays and bomb calorimeter determinations.

Methods of analysis

1. The thiamine determinations were made with the fermentometer according to the procedure of Frey, Atkin, and Schultz ('42).

2. Microbiological methods were used for the determination of riboflavin, pantothenic acid, and niacin. These are fully described in The University of Texas monograph, "Studies on the Vitamin Content of Tissues," published under the direction of R. J. Williams ('41). The organisms used were obtained from the Biochemistry Department of The University of Texas.

3. The A.O.A.C. method ('35) was used for calcium assay. Phosphorus was determined colorimetrically by the method described by Koenig and Johnson ('42) using a Fisher electrophotometer.

4. Protein intakes were calculated from nitrogen values obtained by the usual macro-Kjeldahl procedure.

5. Bomb calorimeter determinations were made in the Chemistry Department of The University of Texas, under the supervision of Dr. John Griswold. Determinations were made on six samples from the Latin-American group and four each from the Anglo-American and Negro groups. All these samples with one exception were from the third collection period. The average figure of 5 calories per gram (dry weight) obtained from these analyses was used to calculate the caloric value of the food intakes.

Physical examination

Examination for anatomical evidence of vitamin deficiencies was made by Dr. Norman Jolliffe. The criteria used are summarized by Jolliffe and Stern ('42).

Table 1 presents for the twenty-four women participating the average daily intakes of calories, protein, calcium, phosphorus, and the four vitamins determined for the period or periods during which diets were obtained from the respective individual. The average daily intakes for each racial group and averages for all groups are shown. The vitamins are listed for which anatomical evidence of deficiency was discovered.

Riboflavin

Of all the women studied the average daily intake was 0.78 mg. per day, which is less than one-half the recommended daily allowance of 1.8 mg. for the sedentary woman. (Observation of the limited physical activity of the subjects led to their classification as sedentary.) In only one case (Subject B in the Negro group) did the intake equal the recommended allowance, but in no instance did it fall below 0.5 mg. (approximately one-fourth of the requirement). For nineteen out of the twenty-four women studied the average intake fell between one-fourth and one-half of the recommended allowance. This finding is emphasized by the incidence of cheilosis, corneal vascularity, or skin lesions in seven of the fifteen subjects examined by Dr. Jolliffe.

Pantothenic acid

The average intake of pantothenic acid was 2.41 mg. per day, with small variation for the three different groups. There is no "recommended allowance" for pantothenic acid, but an allowance of 10 mg. per day has been suggested by R. J. Williams ('42). The highest intake recorded in the present study was 4.15 mg., which again was for Subject B in the Negro group. A study of the data indicates that thirteen of the twenty-four subjects were receiving one-fourth to one-half the intake suggested as adequate.

TABLE 1

Average daily intakes and nutritional status of individual women

	NO. OF DAYS	ENERGY [1]	PROTEIN [2]	CALCIUM	PHOSPHORUS	RIBOFLAVIN	PANTOTHENIC ACID	NIACIN	THIAMINE	ANATOMICAL EVIDENCE OF DEFICIENCY IN [3]
		Cal	gm	gm.	gm.	mg.	mg	mg	mg.	
Anglo-American										
C	14	848	21.63	0.19	0.63	0.55	2.41	3.28	0.33	....
L	14	1160	28.21	0.38	0.93	0.91	2.59	3.06	0.45	Riboflavin
G	14	1198	26.03*	0.27	0.72	0.52	2.36	4.22	0.43	....
Ha	14	1610	31.08	0.45	1.22	0.74	3.06	5.70	0.75	Riboflavin, niacin, ascorbic acid
Hu	7	900	30.11	0.17	0.77	0.73	2.49	2.86	0.41	Vitamin A, niacin, ascorbic acid
S	7	1785	....	0.52	0.95	0.93	2.58	6.25	0.51	Vitamin A
W	7	915	....	0.40	0.38	0.95	2.79	5.25	0.47	....
Ku	7	1465	45.36	0.40	0.90	0.83	2.85	7.65	0.66	None
T	7	1045	24.62	0.28	1.06	0.56	2.14	3.40	0.59	....
De L	7	1435	45.66	0.48	1.98	0.88	3.58	4.01	0.56	Riboflavin, A, niacin, ascorbic acid
Kn	7	1370	41.43	0.51	1.37	0.81	3.11	4.41	0.60	Riboflavin, niacin
Average		1248	32.68	0.36	0.96	0.74	2.69	6.40	0.51	
Latin-American										
E	21	1230	31.15*	0.51	0.73	0.74	1.98	2.80	0.42	Niacin and ascorbic acid
F	21	863	26.93*	0.27	0.34	0.55	1.67	2.71	0.32	Riboflavin, niacin, ascorbic acid
G	21	1565	41.47*	0.55	0.55	0.72	2.55	2.78	0.59	....
M	21	1235	35.08*	0.40	0.64	0.59	2.29	3.28	0.45	Ascorbic acid
Ra	7	1315	....	0.50	1.18	0.56	2.34	3.10	0.60	....
Ram	7	1335	48.57	0.47	0.58	0.66	2.66	5.20	0.46	Vitamin A and ascorbic acid
S	7	1200	34.70	0.37	0.62	0.70	2.74	3.28	0.44	Riboflavin, niacin
Average		1249	36.32	0.44	0.61	0.65	2.21	3.09	0.45	
Negro										
B	21	1805	55.44*	0.65	0.82	1.80	4.15	9.76	0.92	Ascorbic acid, vitamin A
H	21	892	25.93*	0.25	0.56	0.62	1.77	3.21	0.42	Vitamin A, riboflavin, niacin
J	21	753	21.48*	0.28	0.27	0.62	1.62	3.80	0.27	....
M	21	728	26.40*	0.27	0.37	0.75	1.72	3.27	0.35	....
N	7	1620	46.59	0.60	0.73	1.02	2.69	5.86	0.82	Niacin, ascorbic acid
W	7	765	18.62	0.25	..	0.58	1.58	2.88	0.33	....
Average		938	32.41	0.36	0.59	0.95	2.34	4.91	0.51	
Average for all groups		1145	33.80	0.38	0.72	0.78	2.41	4.13	0.49	

[1] Calculated on dry weights from an average value of 5 calories per gram obtained by bomb calorimeter determinations on representative samples.

[2] Starred values represent average of two periods of study; others one period.

[3] Examinations made by Dr. Norman Jolliffe on fifteen subjects.

Niacin

The average intake of niacin for all subjects was 4.13 mg. per day, with the intake of the Latin-American group definitely below the other two. Again Subject B in the Negro group had the highest intake found in this study, 9.76 mg. as compared to the recommended daily alowance of 12 mg. Three of the women had average intakes equal to more than one-half of the recommended allowance, and nineteen had average intakes above one-fourth of this amount. Nine of the fifteen subjects examined by Dr. Jolliffe showed the milder signs of niacin deficiency, such as edema and lateral redness of the tongue and red swollen papillae.

Thiamine

The recommended allowance for thiamine is 1.2 mg. per day. The average intake for the twenty-four women was 0.51 mg. per day. The highest daily intake recorded was 0.92 mg. for Subject B in the Negro group. Of the twenty-four average intakes presented eight are equal to one-half or more of the allowance and only five are less than one-third. Of the fifteen women examined by Dr. Jolliffe none showed diagnosable polyneuritis, but two showed plantar dysesthesia, which is the earliest objective evidence of polyneuritis.

While the diets studied were not assayed for vitamin A or ascorbic acid, the evidences for deficiency of these vitamins found by Dr. Jolliffe are included. Six subjects showed gross Bitot's spots, and all the remaining subjects had abnormal conjunctivae characteristic of xerosis. Nine women showed marginal gingivitis.

Calcium and phosphorus

A study of table 1 shows rather large variations in the calcium and phosphorus intakes of the individual women, ranging from 0.17 to 0.65 gm. for calcium and 0.23 to 1.98 gm. for phosphorus, with intakes of calcium and phosphorus well below recommended allowances. Eleven women had calcium

intakes less than one-half the recommended allowance of 0.8 gm. per day, and twelve had intakes from one-half to three-fourths the allowance. Two women had phosphorus intakes over the recommended allowance of 1.32 gm. Ten were below one-half the allowance. Nine of the remaining received one-half to three-fourths the allowance daily.

Protein

The average intake of protein for all the women studied was 33.80 gm. protein daily. Considerable variation in individual intake was encountered, the values ranging from 19 to 60 gm. The majority of the women apparently received from one-half to two-thirds the recommended protein allowance of 60 gm. per day. The average intake for each group and for all groups was below the amount estimated by Sherman ('41) as the minimum necessary for nitrogen balance in the adult man (44.4 gm.).

Calories

The caloric intake calculated as described above was surprisingly low for all groups. Individual intakes showed considerable variation, ranging from 620 to 2050 calories. The average for all the women was 1145 calories. When the intakes were compared with the standard allowance of 2100 calories for the sedentary woman, it was found that one-third of the women were consuming less than one-half the intake considered adequate, with half receiving from one-half to three-fourths the recommended allowance.

In order to indicate the extent to which the marked calorie deficiency might be correlated with the low protein, mineral, and vitamin values, the average intakes of these nutrients for each group were calculated to a 2100 caloric basis. These calculations indicated that a consumption of enough of the same kind of food as was eaten by the women cooperating in this study would have meant nearly adequate intakes of protein, phosphorus, and calcium and great improvement in vitamin intakes. If 2100 calories had been consumed the average

intakes of thiamine and riboflavin would have been approximately 75% adequate, niacin 62%, and pantothenic acid 43%.

Group differences in intake

On the whole the data show no great differences in the vitamin intakes of the three groups. The average intakes for the twenty-four women studied were slightly more than one-third the recommended allowance for thiamine, niacin, and riboflavin and one-fourth the suggested allowance for pantothenic acid. Phosphorus intakes more nearly approached the recommended allowance than did the calcium intakes, except in the Latin-American group. The calcium deficiency was more marked than the phosphorus deficiency in both the Anglo-American and Negro groups. The calcium intake for the Latin-American group was 25% greater than that of the other two groups, and the phosphorus intake was 50% greater in the Anglo-American than in the other groups. Very little difference was shown in the average daily protein intake of the three groups. The average caloric intakes for the Anglo-American and Latin-American groups were the same and were about 60% of the recommended allowance. The average intake for the Negro was 25% less than for the other groups and only 45% of the recommended allowance.

Seasonal variation

With the intention of discovering seasonal variation diets were collected from the Anglo-American women in November, February, and April, from the Latin-Americans in December, February–March, and May, and from the Negroes in January, March, and June. Since the make-up of the Anglo-American group varied from period to period, it was impossible to draw valid conclusions. Four of the women in both Latin-American and Negro groups made the collections for the three periods. In all three cases, however, averages for the periods show relatively small variation. This seems to indicate that seasonal availability of various products is a factor of little importance in the food selection of these low-income urban groups.

Physical findings

There is a fairly close correlation between the analytical data and the findings of physical examination except in the case of thiamine where the incidence of mild polyneuritis might have been expected. The absence of striking symptoms may be partially accounted for by the low caloric intake and perhaps by the adjustment of these people over a lifetime to diets uniformly deficient in calories, minerals, protein, and vitamins. Certain significant, although non-specific, symptoms were reported, twelve of the fifteen women examined stating that they were nervous or suffered with "nerves." Six experienced frequent headaches, and eight stated that they were easily fatigued.

DISCUSSION AND CONCLUSIONS

The method of computing intake of nutrients by analyzing a duplicate of the food consumed has not so far as is known, been used previous to the present investigation. The commonly used methods of ascertaining dietary intake of individuals are (1) analysis of aliquots of a weighed diet, and (2) computations from food-intake records. It was not possible to use the first of these in the present study and it was thought that the assay of duplicates possessed marked advantages over diet-record computations since it involves food as actually consumed and avoids the many errors inherent in the use of laboratory hand-book figures. Exact duplicates of intake cannot, of course, be obtained without weighing every portion, but if the collector is conscientious the errors involved over a week's period tend to cancel each other and the duplicate represents actual intake with reasonable accuracy. This was proved by collecting samples both with and without weighing. The chief source of error lies in lack of care in collecting the duplicate. The possibility that women in a low-income group would be tempted to collect smaller portions than were actually consumed suggests itself. Observation of and acquaintance with the women participating in this study indicate that small

samples were not due to "cheating" but to poor physical condition, lack of appetite and, especially, to unpalatable food. The samples were always called for by the investigators themselves and the women were questioned when the samples were particularly small. Minor illnesses and lack of appetite were the usual reasons given. Only in the case of milk was the economic factor mentioned. The mothers bought milk for the children, but could not afford it for themselves. The economic factor was, of course, indirectly involved since it determined, in general, the kind of food bought. Table 2 lists the foods consumed by the six women having the lowest calorie intake and makes evident the unattractiveness and monotony of the daily diet. Moreover, food preparation was almost invariably poor. Whether as a cause or result of the low-calorie intake, the restricted activity of the women is a factor worthy of comment. In general, their housekeeping standards were low and their outside activity negligible. Conversation with the women revealed the following picture of their activities and food habits:

The mother gets up fairly early in the morning and prepares breakfast for the rest of the family. She does not eat her own breakfast until after the husband has gone to work and the children to school. By this time she is too tired to eat very much and often goes back to bed for additional rest. Lunch is only a snack since the husband and children are away and no regular meal is prepared. At night, after preparing dinner for the family, the mother is again too tired to eat normally.

When the first assays were completed and the low figures became apparent, efforts were redoubled to make sure that complete samples were obtained. The women were encouraged to report any item for which a duplicate had not been available, e.g., an egg, glass of milk, slice of bread, etc. so that it might be secured and added to the diet.

In comparison with the recommended allowances the intakes reported are unusually low and the correlation between intakes and physical condition is not as high as might be expected. The intakes are also lower than are reported in other studies of low-income groups. However, most of these other studies have been done on the family group as a whole and results re-

TABLE 2

Typical daily food lists of low-income women [1]

Subject M — Negro
1. Cornmeal mush, prunes, string beans, potatoes, white bread.
2. Cabbage, hamburger, white bread.
3. Potatoes, carrots, roll.
4. Cornmeal mush, string beans, carrots, cauliflower, cornbread, toast.
5. Rice, ham, white bread, kidney beans, vegetable salad, cornbread, cake.
6. Rolls, fresh peas, macaroni, ham, omelet.
7. Rice, white bread, cabbage, fresh peas, hamburger, cornbread.

Subject J —Negro
1. Grits, lettuce, potatoes, rolls, gravy.
2. Lima beans, white bread, grits, prunes.
3. Cornbread, weiners, applesauce, macaroni and cheese.
4. Dried beans, sweet potatoes, cornbread, grits, white bread.
5. Spinach, potatoes, hamburger, cornbread, grits, white bread.
6. Greens, cornbread, potatoes, oatmeal, white bread.
7. Potatoes, lettuce, bacon, white bread, oatmeal, milk, white bread.

Subject H — Negro
1. Cabbage, meat, potatoes, cake, peas, white bread, tomatoes, cereal, prunes, milk.
2. Beans, prunes, white bread, crackers, spinach, potatoes, grits.
3. Cabbage, peas, potatoes, salmon, white bread, crackers, cake, cereal.
4. Potatoes, tomatoes, white bread, peanut butter, crackers, cereal.
5. Soup, potatoes, crackers, white bread, cereal, milk.
6. Beans, potatoes, sardines, white bread, cereal.
7. Cabbage, salmon, potatoes, white bread, peanut butter, cereal.

Subject F — Latin-American
1. Cream of wheat, beans, cheese, onions, white bread, jelly, chili con carne.
2. Bacon, egg, enchilada, meat, potatoes, cocoa.
3. Oatmeal, cookie, white bread, corn, lemon pie, beans, gravy, milk.
4. Oatmeal, toast, enchilada, beans, crackers, peaches, spinach, eggs, white bread, milk.
5. Egg, toast, potatoes, white bread, peanut butter, orange juice, pork and beans, green peppers, egg. white flour tortillas.
6. Egg, toast, cocoa, bread, crackers, potatoes, hamburger, tortillas, beans.
7. Egg, bread, gravy, milk, beans, crackers, mayonnaise.

Subject Hu — White
1. Potatoes, bacon, biscuit, raisins, cornbread, chili, crackers, beans, oatmeal.
2. Cornbread, vegetable soup, white bread, beans, raisins, oatmeal, biscuits.
3. Sausage, white bread, gravy, lettuce, bacon, fresh peas, fresh peaches, honey, oatmeal.
4. Potatoes, peas, gravy, bacon, bread, hot cakes, syrup, biscuit, oatmeal.
5. Dried beans, cornbread, potatoes, sweet potatoes, dried peas, boiled pork, lettuce, white bread, oatmeal, milk.
6. Potatoes, bacon, white bread, chocolate custard, sweet potatoes, soup.
7. Egg, apple, white bread, bacon.

Subject C — White
1. Potatoes, macaroni, pickles, white bread, jello.
2. Dried beans, vegetable soup, crackers, stewed apple.
3. Spaghetti, crackers, egg, toast.
4. Dried beans, potatoes, bread pudding, okra and tomatoes, crackers, white bread.
5. Potatoes, okra and tomatoes, bread, peanut butter, crackers.
6. Potatoes, white bread, liver, gravy.
7. Potatoes, crackers, tomatoes.

[1] Coffee, sugar, and butter or oleomargarine also appear on the daily diets, but are not recorded.

ported on a "unit" basis. Hunter and Pett ('41) in a dietary survey of 76 families in which intakes for each member of the family were separately calculated concluded that food was not fairly distributed in the family and that the worst fed member was the mother. They state that 78% of the women were deficient in calories and only 42% of the men. Similar figures for calcium are 66% in comparison with 28%, for iron 95% with 5% and vitamin A 80% with 52%. Actual figures on intake are not given, except the average for the group as a whole, but it was shown that more of the families on a yearly income of $500–$1000 were deficient than of the families on incomes of $1000–$1500. Families in the study here reported were within the $500–$600 income range.

Milam ('42) in a dietary survey of a small North Carolina community, stated that the average calorie intake of that portion of the community over 15 years of age was 2000. This corresponded closely with the intake of a group of doctors and technicians at Duke Hospital. For only part of his study does he give separate calorie intakes for women and these are stated to be 1577 for whites and 1443 for Negroes.

Youmans et al. ('43) in a study of a rural population in Tennessee obtained calorie intake figures that are somewhat more comparable to those reported here. Calories were calculated from a 3-day intake record. The group studied comprised 776 whites and 385 colored. Included in this group were 229 adult white and 96 adult colored females. Of this total group of 325, 29.1% had calorie intakes below 1250; in the white group the percentage was 18.6% and in the colored 54.9%. Youmans points out that the recorded calorie intakes are not only relatively low but absolutely so low that their continuance for any but the shortest periods would seem inevitably to be followed by disabling loss of weight and strength. Weight records, however, showed that only 5% of the colored women were underweight and 10% of the white. In other words a larger percentage of the colored than the white women had calorie intakes below 1250, but a much smaller percentage were underweight. A more intensive study was made on a representative

10% of the group and it was shown that as many as one third of the group had intakes less than 30% above actual basal requirements while several had intakes that were below. Moreover, only a small part of this group were as much as 20% below their predicted "ideal" weight and many were over that weight by as much as 30%.

Very few of the group represented in the present investigation were underweight and several were considerably overweight. Calorie intake figures obtained in the present investigation tend to confirm the lack of correlation noted by Youmans between estimated calorie intake and body weight. Results obtained by the present investigators in a further study (to be presented later) in which diet samples were collected from a higher income group and from a group in which the collection of samples was done with special care, are similar to those obtained in the present study. Moreover, the large number of caloric intakes below 1250 found by Youmans is in accord with the findings of the present study of an average of 1248 for the white, 1249 for the Latin-American, and 938 for the Negroes. Four of the six Negro women studied had exceptionally low intakes. Youman's figures on protein, mineral, and vitamin intake have not yet appeared, but it is probable that low-calorie intakes will be accompanied by low intakes of other factors. Youmans raises the question of whether the recommended allowances have been placed too high, and it would seem that enough data have accumulated to make this possibility worthy of further investigation.

SUMMARY

The study of three hundred eight separate diets collected from twenty-four women of the low-income group for weekly periods at three different seasons revealed severe deficiencies in calories, protein, minerals, and vitamins. When the average daily intakes were compared with the daily allowances for the sedentary woman recommended by the Committee on Food and Nutrition of the National Research Council, it was found that the calorie intake was from one-half to three-fourths the al-

lowance, the average intakes of thiamine, niacin, and riboflavin, slightly more than one-third, and protein, calcium, and phosphorus approximately one-half the amounts recommended as adequate. The pantothenic acid intake was about one-fourth the amount suggested by R. J. Williams ('42) as representing adequacy for this vitamin. Seasonal variation and differences between the intakes of Anglo-American, Latin-American, and Negro subjects were found to be slight.

The findings of physical examination of fifteen of the twenty-four women corroborate the data obtained from the dietary assay for the B-complex of vitamins. The fact that no extreme cases of deficiency were observed and that all cases did not show the same degree of deficiency on similar intakes does not alter the conclusion that among people of the low-income group represented in this study chronic nutritional deficiency is prevalent. On the other hand the absence of grave manifestations of malnutrition on the low intakes found, and particularly the failure to observe underweight as a result of the low calorie intakes, suggests the possibility that the recommended allowances have been placed too high. Reference is made to other investigations showing similar results.

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RIBOFLAVIN AND THIAMINE INTERRELATION-
SHIPS IN RATS AND IN MAN [1]

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ONE FIGURE

INTRODUCTION

Rats markedly deficient in thiamine have been found to
deviate somewhat from normal in their metabolism of ribo-
flavin. Supplee et al. ('42) observed that after the ingestion
of food there is a less than usual increase in liver riboflavin
in thiamine deficient animals. Sure and Ford ('42) found
that the concentration of riboflavin in the tissues of rats
suffering from severe thiamine deficiency was 10 to 20%
less than that of their control animals. The suggestion
of the latter authors that thiamine deficiency, by affecting
riboflavin retention and utilization, may be of clinical sig-
nificance in the production of riboflavin deficiency in man,
has occasioned the present review of data which had been
collected in the course of another investigation (Ferrebee,
Weissman, Parker and Owen, '42).

METHODS

The details of the experimental procedures and the
methods of analysis have been described elsewhere (Ferre-
bee, Weissman, Parker and Owen, '42; Ferrebee, '40 a, b;
Ferrebee and Carden, '40). Male white rats of the Wistar

strain, weighing 180 gm., were placed on a thiamine deficient diet (Ferrebee, Weissman, Parker and Owen, '42). The concentrations of riboflavin and thiamine in their livers and kidneys were compared at intervals of 1 to 4 weeks with the concentrations observed in control animals which received the thiamine deficient diet with either unautoclaved yeast or thiamine.

Measurements were made of riboflavin and thiamine excretion in the control and in the deficient animals, both during fasting and after the administration by various routes of known amounts of riboflavin and thiamine. All animals were fasted overnight before they were killed for tissue analyses or used for excretion tests. During the excretion tests animals were fasted, but allowed water ad libitum.

In a group of patients of varying nutritional status, measurements were made of tissue riboflavin and thiamine concentrations and urinary thiamine and riboflavin excretion. The excretion experiments were performed in the morning on fasting patients who had received 200 to 400 ml. of water ½ hour before the injection of vitamin. The measurements of vitamin excretion were accompanied by measurements of blood urea and of urinary urea excretion.

<div align="center">RESULTS</div>

<div align="center">*A. Experiments on rats*</div>

Figure 1 illustrates the effect of a thiamine deficient diet upon the growth curve of 180 gm. male Wistar rats (Ferrebee, Weissman, Parker and Owen, '42). Isocaloric feeding does not maintain parallelism in growth in the control and thiamine deficient animals (Sure and Dichek, '41; Sure and Ford, '42). No effort was made to secure equal caloric intakes in our control and thiamine deficient groups. During the third and fourth week of the experiment the deficient animals lost weight and their livers were found to be small and the capsule shrunken and white.

Table 1 presents data on the concentration of riboflavin and thiamine in the livers and kidneys of a group of four-

teen control and fourteen thiamine deficient animals. Thiamine concentrations fell to critical levels in about 2 weeks. Weight loss begins at this time and apparently delays further fall in thiamine concentrations (Ferrebee, Weissman, Parker and Owen, '42).

In general the concentration of riboflavin in the livers of our thiamine deficient animals is lower than that observed in the control group. However, judging from the variations observed in both groups, this difference is not of great phy-
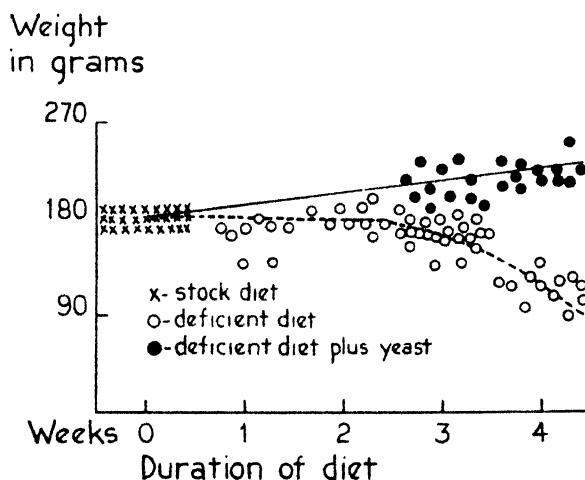
Fig. 1   Effect of thiamine deficient diet on growth curve of 180 gm. male Wistar rats.

siological or clinical importance. Furthermore, it is evident that progressive thiamine deficiency does not cause a progressive fall in tissue riboflavin concentrations. The liver riboflavin concentrations at the end of 4 weeks of thiamine deficiency are essentially equal to those observed in the earlier periods of deficiency. The differences in kidney riboflavin concentrations in the control and in the thiamine deficient groups throughout the experiment appear scarcely worthy of comment.

Table 2 presents data on the excretion of riboflavin and thiamine in eleven control animals and in eleven animals

that had been on a thiamine deficient diet for a period of 2 weeks. It may be seen that the individual variations in the excretion of riboflavin exceed the differences which may be detected between the control and the thiamine deficient group. For comparison, data are also given from a num-

TABLE 1

Riboflavin and thiamine concentrations in livers and kidneys of thiamine deficient rats.

(Concentrations expressed in micrograms per gram of fresh tissue.)

DURATION OF DIET	RIBOFLAVIN IN LIVER		RIBOFLAVIN IN KIDNEY		THIAMINE IN LIVER		THIAMINE IN KIDNEY	
	Control	Deficient	Control	Deficient	Control	Deficient	Control	Deficient
3 days	20	23	20	23	9	4	4	3
	20	29	21	27	10	6	5	3
	20	27	19	25	10	5	5	3
8 days	31	21	24	26	12	2	6	1
	36	26	20	28	9	2	5	1
	28	23	25	24	11	2	6	1
2 weeks	21	27	21	24	8	1	5	1
	30	26	21	25	9	1	4	1
3 weeks	33	24	31	24	11	1	5	1
	23	24	32	24	8	1	3	1
	35	22	33	22	9	1	6	2
	40	24	27	27	8	1	5	1
4 weeks	35	20	29	25	10	1	5	1
	36	26	39	25	11	1	7	1
Average of 3 and 4 week periods.	34	23	32	25	10	1	5	1

ber of tolerance tests for riboflavin and thiamine excretion performed on a similar group of animals after subsisting for 2 weeks on a thiamine deficient diet. The data of table 2 give little indication that thiamine deficiency of 2 weeks' duration affects riboflavin excretion.

TABLE 2

Urinary excretion of riboflavin and thiamine by rats which have been on a thiamine-deficient diet for 2 weeks: Basal excretion, and excretion after small test quantities of riboflavin and thiamine.

DURATION OF URINE COLLECTION	RIBOFLAVIN EXCRETED (MICROGRAMS)		THIAMINE EXCRETED (MICROGRAMS)		TYPE OF EXCRETION TEST
	Control	Deficient	Control	Deficient	
24 hrs.	66	28	4	1	
	51	49	3	1	Basal excretion: thia-
	42	26	3	1	mine deficient diet for
	34	38	2	1	2 weeks.
Average	43	35	3	1	
3½ hrs.	4	10	0.6	0.3	
	4	7	0.6	0.3	Basal excretion: thia-
	9	9	0.7	0.3	mine deficient diet for
	8	8	0.9	0.3	2 weeks.
Average	7	9	0.7	0.3	
1½ hrs.	3	1	0.3	0.1	
	5	3	0.6	0.2	Basal excretion: thia-
	4	3	0.3	0.2	mine deficient diet for
Average	4	2	0.4	0.2	2 weeks.
24 hrs.	46	36	10	2	
	48	40	9	1	160 μg. riboflavin and
	48	65	7	8	thiamine per os in 4
	42	25	6	2	ml. normal saline.
Average	46	42	8	3	
3½ hrs.	21	27	7	0.5	
	43	23	16	0.4	40 μg. riboflavin and
	20	22	6	0.5	thiamine injected sub-
	..	22	..	0.5	cutaneously in 1 ml.
Average	28	23	10	0.5	normal saline.
1½ hrs.	5	20	5	2	
	15	20	9	3	40 μg. riboflavin and
	15	21	13	5	thiamine injected in-
Average	12	20	9	3	travenously in 1 ml. normal saline.

B. Experiments on man

Table 3 presents data on the concentrations of riboflavin and of thiamine found in the tissues of presumably "normal" individuals, compared with the concentrations found in the tissues of individuals with "poor" nutrition. The clinical material is not sufficient to permit generalizations, but one or two comments may be pertinent.

TABLE 3

Concentrations of riboflavin and thiamine in human tissues.
(Micrograms per gram of fresh tissue.)

SUBJECT	AGE IN YEARS	SEX		HEART	SKELETAL MUSCLE	LIVER	KIDNEY CORTEX	CEREBRAL CORTEX	REMARKS
Presumably "normal" individuals.	10	M	Riboflavin	8.7	4.0	15.7	16.1	..	Food in stomach, traumatic death
			Thiamine	3.5	1.2	1.4	2.1	..	
	30	M	Riboflavin	6.4	1.6	14.1	11.8	2.2	Negro, traumatic death
			Thiamine	2.4	0.4	1.0	1.8	1.1	
	52	M	Riboflavin	5.4	1.2	9.9	10.1	1.6	Traumatic death, survived 24 hours
			Thiamine	2.0	0.4	1.2	1.3	0.8	
Patients with "poor" nutrition.	34	F	Riboflavin	6.9	1.2	8.3	9.1	..	High cord injury, sepsis 3½ months
			Thiamine	0.9	0.2	0.7	1.0	..	
	38	F	Riboflavin	4.8	1.3	3.2	4.8	1.1	Active tuberculosis of spine and adrenals, high fever
			Thiamine	0.6	0.0	0.3	0.4	0.5	
	49	F	Riboflavin	6.4	1.5	11.6	3.6[1]	..	Lymphosarcoma, uremia, high fever for 3 weeks
			Thiamine	0.5	0.1	0.6	0.3[1]	..	

[1] Kidney infiltrated with tumor.

The tissue analyses (table 3), show that only one of the patients with "poor" nutrition was deficient in riboflavin. This was the patient with tuberculosis of the spine and adrenals, who had been anorexic for some weeks and had been maintained on glucose and saline infusions during a terminal febrile illness of 2 weeks' duration. In this individual the concentrations of riboflavin in the liver and in the kid-

neys were considerably less than normal. The concentrations of riboflavin in the heart and in the skeletal muscles, on the other hand, were essentially normal for her age group. The relatively normal concentration of riboflavin in the muscles of patients thought to have riboflavin deficiency has received previous comment (Axelrod, Spies and Elvehjem, '41).

The data on both riboflavin and thiamine concentrations (table 3) reveal that the two other individuals with "poor" nutrition suffered from thiamine deficiency but not from riboflavin deficiency. In other words, their riboflavin nutrition was not affected by a rather considerable degree of thiamine deficiency.

In a group of fourteen determinations of the simultaneous excretion of riboflavin and thiamine, it was found in eight normal individuals that following intravenous injection of 1 mg. of each of the vitamins, 20 to 25% of the thiamine injected, and 30 to 35% of the riboflavin, appeared in the urine within 1 hour (Ferrebee and Carden, unpublished data). The values for the 2-, 3-, and 24-hour excretions following this type of test were in agreement with the observations reported by Najjar and Holt ('41) and will not be given in detail.

Table 4 presents data obtained when the 1-hour intravenous test was applied to a group of four patients suspected of dietary deficiency. Blood urea concentrations in this group were normal (Najjar and Holt, '40).

It may be observed that essentially normal values for both riboflavin and thiamine excretion were obtained in patients "Bz" and "Cy" and that a greater than usual excretion of riboflavin and thiamine was observed after several days of vitamin therapy.

Excretion tests indicated that thiamine deficiency was present in the two other patients, "Ns" and "Br," and this thiamine deficiency again did not seem to be accompanied by evidences of riboflavin deficiency (Najjar and Holt, '41).

The excretion of thiamine by patient "Ns" was less than normal on two occasions and the abnormality was not associated with abnormality of general renal function, that is, urea, water, or riboflavin excretion. The excretion of urea

TABLE 4

Per cent of administered vitamin excreted in 1 hour following intravenous injection of 1 mg. of riboflavin and 1 mg. of thiamine in four subjects suspected of dietary deficiency.

PATIENT	DATE	STATUS	MILLI LITERS OF URINE EXCRETED	GRAMS OF UREA EXCRETED	PER CENT OF RIBO-FLAVIN TEST EX-CRETED	PER CENT OF THIAMINE TEST EX-CRETED
Bz	6-15-40	Anorexia nervosa	295	..	26	21
	6-17	Anorexia nervosa	282	.62	26	19
	6-24	10 mg. B₁ and B₂ for 5 days	537	1.10	44	33
Cy	6-15-40	Alcoholic polyneuritis	110	..	24	21
	6-17	Alcoholic polyneuritis	51[1]	.43[1]	17[1]	15[1]
	6-24	Yeast tablets daily for 1 wk.	325	1.50	52	30
Ns	6-15-40	Diet low in B complex	175	..	25	13
	6-17	Diet low in B complex	170	.65	23	12
	6-24	10 mg. B₁ and B₂ for 5 days	105	.68	39	27
Br	7-2-40	Congestive heart failure, ? thiamine deficiency	250	1.70	46	9

[1] Specimens probably incomplete.

and water was unchanged by vitamin therapy; but the thiamine excretion returned to normal, indicating that the previous low values for thiamine excretion were related to the suspected thiamine deficiency. The excretion of thia-

mine by patient "Br" was considerably less than normal, the values for water, urea, and riboflavin excretion being at the same time elevated by a moderate diuresis caused by bed rest and cardiac therapy.

DISCUSSION

The observations reported fail to demonstrate that thiamine deficiency per se has an effect upon riboflavin metabolism. Animals maintained from infancy on markedly deficient diets (Supplee, Jensen, Bender and Kahlenberg, '42) or maintained in thiamine deficiency to the point of death (Sure and Ford, '42) may show some slight deviations from normal in their tissue riboflavin concentrations or in their urinary riboflavin excretion. These deviations are by no means clinically impressive and their occurrence is not particularly surprising in view of the extreme degree of secondary malnutrition and general marasmus exhibited by the animals in question.

The observation that thiamine deficient animals in the later stages of deficiency lose weight rapidly is ample explanation for the increased excretion of riboflavin observed in that period (Sure and Ford, '42). Our observations indicate that an increased excretion of riboflavin is not observed in the earlier stages of thiamine deficiency before weight loss is prominent, despite the fact that tissue thiamine concentrations at this time have reached levels comparable to those maintained in the later stages of deficiency (Ferrebee, Weissman, Parker and Owen, '42). The increased excretion of riboflavin appears therefore to be an unspecific phenomenon' of the late stages of thiamine deficiency related to the rapid metabolism of tissues that occurs when the thiamine requirements can be satisfied only by tissue loss. The shrinkage of the liver observed in this period is sufficient in itself to explain the origin of a great part of the riboflavin that appears in the urine at this time. Excretion of this riboflavin, representing as it does an excretion of some of the unused products of tissue catabolism

(Sure and Dichek, '41) can scarcely be taken as evidence of riboflavin deficiency. In fact, it might be argued that in so far as tissue catabolism makes riboflavin, as well as thiamine, available from tissue sources, the dietary requirements for riboflavin may be reduced during periods of severe thiamine deficiency. The common observation that evidences of multiple or secondary deficiencies frequently make their appearance during the curative phase of primary deficiencies, rather than during the acute phase of these deficiencies, is corroborative of this view. This suggests as a corollary that the dietary requirements for riboflavin and other vitamins may be increased during the period of weight gain and tissue reconstitution which follows thiamine therapy. Vitamin interrelationships of this type have been illustrated and discussed by Morgan ('41).

As for the suggestion that thiamine deficiency may be of significance in the production of riboflavin deficiency in man, it may be observed that the above arguments are applicable to this question and that clinical investigation has failed to disclose an obligatory association of the two deficiencies. In the writers' experience it has been difficult to obtain objective evidence that thiamine deficiency, either clinical or subclinical, is of frequent occurrence in the population we have studied. Tissue analyses and excretion experiments in those few instances in which thiamine deficiency has been demonstrable have not shown that deficiency of this vitamin has an effect upon riboflavin metabolism. In patients whose vitamin nutrition is generally "poor," the necessity for distinguishing riboflavin deficiency due to inadequate riboflavin intake from riboflavin deficiency due to a presumed relationship to thiamine deficiency is at once apparent. Thus it would seem reasonable at the moment to consider that our one patient with evidences of both riboflavin and thiamine deficiency (the patient with tuberculosis of adrenals—table 3), suffered an inadequate intake of both riboflavin and thiamine during the period of her maintenance on glucose infusions. In our four other patients with thia-

mine deficiency neither tissue analyses nor measurements of riboflavin excretion gave evidence that thiamine deficiency had affected their metabolism of riboflavin.

CONCLUSION

Thiamine deficiency is not of clinical significance in the production of riboflavin deficiency. Changes in riboflavin metabolism are observed only in the terminal stages of thiamine deficiency and appear to be unspecific and of minor importance.

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SOME OBSERVATIONS ON ABRINE [1]

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The substance, abrine, was isolated by the Indian workers, Ghatak and Kaul ('32), in the course of a chemical study of the seeds of Abrus precatorius, a plant of the natural order Leguminosae. The seeds (known as jequirity seeds) are common in tropical countries and have been an article of Hindu materia medica from a very remote period. Abrine was found to occur free in the seeds from which it could be extracted by appropriate solvents. The investigators in India described certain tests for the crystalline material which they obtained, but it remained for the Japanese investigator, Hoshino ('35), to reveal that abrine was chemically related to tryptophane. Cahill and Jackson ('38) have shown that this naturally occurring N-methyltryptophane belongs to the same configurational series as $l(-)$-tryptophane.

We thought it of interest to determine whether or not the methyl group of abrine, like the methyl group of methionine, was labile in the sense that it might be readily transferable in vivo and thus render abrine lipotropic under certain conditions. In a second experiment we sought to determine whether abrine could prevent the development of cataract in rats maintained for a prolonged period on a tryptophane-deficient diet.

Abrine for the present study was prepared by a procedure involving extraction of the ground seeds of Abrus precatorius with methanol, as described in detail by Cahill and Jackson ('38). It should be pointed out that a toxic protein, abrin, occurs in the seeds and a mask should be worn while they are being ground. With regard to the toxicity of abrin it has been stated that the "smallest particle in slightest wound may prove fatal." (Merck Index, '40.) Owing to similarity in spelling, abrine is unfortunately sometimes confused with abrin in the literature. The substance, abrine, has no apparent untoward effect on rats to which it is fed.

One sample of abrine employed possessed a specific rotation of $+ 45.3°$ at 25°C. in 0.5 N HCl, another a rotation of $+ 45.7°$. Values of $+ 46.0°$ (Cahill and Jackson, '38) and $+ 44.4°$ (Hoshino, '35) have been reported.

Fatty liver experiments

In the study of the possible lipotropic action of abrine, the experimental procedure described by Tucker and Eckstein ('37) in their investigation on the lipotropic activity of methionine was followed. Male white rats of the Sprague-Dawley strain, weighing approximately 120 to 145 gm., were fed the diets shown in table 1 for 19 days, at the end

TABLE 1

Diets employed in fatty liver experiments.[1]

DIET	I	II	III	IV
	%	%	%	%
Casein	5.0	5.0	5.0	20.0
Glucose	48.0	47.5	47.3	33.0
Agar	2.0	2.0	2.0	2.0
Lard	40.0	40.0	40.0	40.0
Salts (Wesson)	5.0	5.0	5.0	5.0
dl-Methionine	...	0.5	...	...
l(+)-Abrine	...	...	0.73	..

[1] The diet of each experimental animal was supplemented daily with 400 mg. of dried yeast and 2 drops of cod liver oil.

of which time they were decapitated and the total fatty acids in the liver determined essentially according to the method described by Leathes and Raper ('25). The results of this study are shown in table 2. As can be seen in table 2, the livers of the animals fed a 20% casein diet, or a 5% casein diet supplemented with methionine, contained much less fat than the livers of the animals receiving abrine. The amount of abrine fed in diet III was comparable on a molar basis with the supplement of methionine in diet II. The fatty acid content of the livers of the animals receiving abrine in the diet was similar to the fatty acid content of the livers of the unsupplemented controls. The results indicate that abrine is not significantly lipotropic.

TABLE 2

Results of fatty liver experiments.

DIET	NUMBER OF RATS USED	AVERAGE DAILY FOOD CONSUMP- TION	AVERAGE WEIGHT OF LIVER	$\frac{\text{LIVER WT}}{\text{BODY WT}} \times 100$	TOTAL FATTY ACIDS IN LIVER AVERAGE AND RANGE
		gm	*gm*		*%*
I	10	6.49	5.87	4.43	24.2 (10.2–38.2)
II	5	6.25	6.15	4.39	14.8 (9.0–22.7)
III	10	6.47	5.61	4.30	22.2 (13.9–34.1)
IV	4	7.03	6.39	3.80	15.6 (12.7–18.2)

It is possible that in the metabolism of N-methyltryptophane the first step may consist in the removal of methyl amine rather than the methyl group alone. Information on this point was sought by incubating abrine, dissolved in a modified physiological salt solution (Krebs, '33), under an atmosphere of oxygen with kidney slices. Abrine belongs to the *l*-configurational series and for this reason human kidney slices were employed after rat kidney slices yielded no positive

result. In this connection, it has been shown that a human kidney slice will deaminate a naturally occurring amino acid as rapidly as it does the unnatural optical isomer, whereas slices of the kidneys of rats and a number of other animals deaminate the *l*-series amino acids at only a fraction of the rate at which they attack amino acids of the *d*-configurational series (Krebs, '33). Tests for methyl amine after incubation even with human kidney slices were, however, inconclusive.

Cataract experiments

It has been shown by Totter and Day ('42) that the inclusion of as little as 0.1% tryptophane in the diets of animals not receiving this essential amino acid will prevent the development of cataract which is observed in the eyes of rats fed a tryptophane-deficient diet for prolonged periods. Excellent descriptions of the cataract have been reported by Totter and Day ('42) and by Albanese and Buschke ('42). In our study three groups of weanling white rats were fed the acid-hydrolyzed casein diet described by Totter and Day ('42). The basal diet was made up of 14.7% acid-hydrolyzed casein,[2] 0.3% cystine, 15.0% sucrose, 42.0% starch, 2.0% agar, 2.0% salt mixture (Wesson, '32), 5.0% cod liver oil, 19.0% Crisco, and B vitamins were furnished by daily supplementation with 250 mg. of yeast.[3] One group received, in addition, 0.1% tryptophane in the diet, another group 0.107% abrine, and a third group served as a control. The duration of the experiment was 90 days. A summary of the results is shown in table 3.

Although the number of animals employed was small, owing to the limited supply of abrine available for these experiments, the results show clearly that $l(+)$-abrine, like $l(-)$-tryptophane, is able to prevent the occurrence of cataract in rats fed a tryptophane-deficient diet. Inclusion of abrine in the diet of tryptophane-deficient rats promoted

[2] We wish to thank Dr. Melville Sahyun of Frederick Stearns and Company for a gift of acid-hydrolyzed casein.

[3] Harris.

their growth. As shown in table 3, however, the growth resulting from supplementation with abrine was not equivalent to that induced by supplementation with a comparable amount of tryptophane. This is in agreement with Gordon ('39) who also observed that $l(+)$-abrine is not as efficient as $l(-)$-tryptophane in the promotion of growth when fed to rats deficient in tryptophane. This investigator has suggested that the conversion of abrine to tryptophane, or to some physiologically available precursor of this amino acid, is not quantitative .

TABLE 3

Results of cataract experiments.

DIET	NUMBER OF RATS USED	AVERAGE WEIGHT AT BEGINNING	AVERAGE WEIGHT AFTER 90 DAYS	NUMBER OF RATS WITH CATARACT AFTER 90 DAYS
		gm.	gm.	
Tryptophane-deficient	6	34.1	30.8 [1]	4 [1]
Tryptophane-deficient plus 0.1% $l(-)$ tryptophane	6	36.0	107	0
Tryptophane-deficient plus 0.107% $l(+)$-abrine	6	36.1	93	0

[1] 4 surviving rats.

SUMMARY

The naturally occurring N-methyltryptophane, $l(+)$-abrine, did not prevent the accumulation of fat in the livers of animals fed a diet deficient in the labile methyl group.

The growth promoting effect of abrine fed to rats on a tryptophane-deficient diet was confirmed.

The development of cataract in rats maintained on a ration deficient in tryptophane was prevented when the diet was supplemented with abrine.

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LOSSES OF B VITAMINS DUE TO COOKING OF FOODS [1]

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Much information has been obtained recently regarding the distribution of the various B vitamins in natural foodstuffs (Waisman and Elvehjem, '41; Lane, Johnson and Williams, '42; Cheldelin and Williams, '42). However, information is less complete regarding the amounts of these vitamins present in foods as they are actually eaten.

In a previous paper (Lane, Johnson and Williams, '42) the losses in thiamine due to cooking were determined for a large number of foods comprising the average American diet. The present investigation deals with similar losses in six other vitamins: riboflavin, nicotinic acid, pantothenic acid, biotin, inositol and folic acid.

EXPERIMENTAL

Selection and sampling of foods

Vegetables, fruits, milk and eggs were purchased in nearby markets in as fresh condition as possible. Meats were, with few exceptions, obtained from a single wholesale butcher so that it was possible in most cases to be assured of freshness of the samples. Fish were bought as frozen commercial products.[3] Perishable foods were kept at 0–5° C. until they were

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[3] Birds-Eye.

prepared for assay. These preparations were made within a few hours after procurement of the samples.

Procedure

The microbiological assay methods developed in the University of Texas Laboratories (Williams, '41) have been employed throughout for each of the six vitamins herein discussed.

All food extracts were prepared by digestion with takadiastase and papain according to the general method described previously (Cheldelin, Eppright, Snell and Guirard, '42). This procedure has proved to be quite satisfactory for preparation of extracts of natural materials, although it has not been confirmed generally for use with cooked foods. It is possible that in certain cases vitamins which have become "bound" by cooking and thus rendered unavailable to microorganisms (or to the reagents used in various chemical tests) may still be available for animal nutrition. This is apparently true for thiamine in some tissues (Lane, Johnson and Williams, '42). Satisfactory animal assays are not available for several vitamins, however, and in the absence of further direct evidence we have chosen to employ the digestion procedure mentioned above. Vitamin losses which appear to be very large may warrant reexamination at a later time.

RESULTS

Tables 1 and 2 contain the details of cooking and the results of the analyses of samples of thirty foods before and after cooking for the six vitamins mentioned above.

Lane, Johnson and Williams ('42) have pointed out the difficulties in estimating the amounts of cooking waters to be used or discarded in the preparation of foods. In an effort to standardize these factors we have included cooking waters and meat juices as part of the cooked samples except in a few cases where the cooking waters were assayed separately and discarded. The values in table 2 therefore tend to represent maximum amounts of the various vitamins present after cooking.

Details of the cooking of various foods.

FOOD	MANNER OF COOKING	TIME OF COOKING	WATER OR FAT ADDED	WEIGHT BEFORE COOKING	EDIBLE AFTER COOKING
Meats		*Minutes*	*gm.*	*gm.*	*gm.*
1.[1] Beef round	Fried in open pan	15	1	40	30
2. Beef liver	Fried in open pan	10–15	1	40	35
3. Beef liver	Fried, covered pan	10	16	94	75
4. Beef heart	Steamed	30	93	190	210
5. Beef heart	Steamed	30	50	100	150
6. Pork loin	Fried in open pan	10–15	0	40	29
7. Pork loin	Fried in open pan	10–15	0	45	28
8. Pork loin	Fried in open pan	10–15	0	68	43
9. Bacon	Fried in open pan	5	0	41	17
10. Bacon	Fried in open pan	3–5	0	22	13
11. Ham	Fried in open pan	15	0	54	30
12. Ham	Fried in open pan	15	0	59	37
13. Veal chop	Fried in open pan	10–15	0	42	30
14. Veal chop	Steamed	30	84	170	180
15. Lamb leg	Roasted, 300° F.	120	0	690	550
16. Mutton shoulder	Steamed	30	66	133	199
17. Chicken leg	Fried in open pan	10–15	0	41	35
18. Chicken leg	Fried in open pan	10–15	5	69	46
19. Chicken breast	Fried in open pan	10–15	0	31	29
20. Chicken breast	Fried in open pan	10–15	0	31	29
21. Salmon	Fried in open pan	20	3	111	97
22. Halibut	Fried in open pan	7–10	2	126	104
23. Halibut	Steamed	30	0	5	5
Vegetables					
24. Beets	Boiled, covered kettle	40	266	133	124
25. Cooking water					140
26. Beets	Steamed	40	190	380	570
27. Beet tops	Steamed	10	20	40	60
28. Cabbage	Steamed	30	25	100.	125
29. Carrots	Steamed	30	25	50	75
30. Cauliflower	Steamed	20	120	120	240
31. Cauliflower	Steamed	30	50	100	150
32. Onions	Fried in open pan	20	29	208	53
33. Potatoes	Boiled, covered kettle	30	200	190	200
34. Cooking water					190
35. Potatoes	Boiled, covered kettle	20	360	180	210
36. Cooking water					210
37. Lima beans	Steamed	60	10	5	15
38. Okra	Steamed	20	15	30	45
39. Rice	Steamed	25	60	30	90
40. Sauerkraut	Steamed	30	0	5	5
41. Spinach	Steamed	10	50	100	150
42. Split peas	Steamed	40	100	50	150
43. Sweet potatoes	Baked	45–60	0	2200	1700
44. Turnips	Boiled, covered kettle	40	114	114	118
45. Cooking water					90
46. Turnips	Steamed	20	22	22	44
Fruit					
47. Apples	Boiled, open kettle	20–30	1300	3400	2300
Eggs and milk					
48. Eggs	Scrambled, open pan	10–15	8	150	139
49. Eggs	Scrambled, open pan	10–15	1	50	46
50. Eggs	Baked	30	0	100	100
51. Eggs	Scrambled, covered pan	5	0	100	94
52. Milk	Heated, glass dish	30	0	100	100
53. Milk	Heated, open metal pan	15	0	100	100

[1] The numbers in this column correspond to those in the first column of table 2.

TABLE 2

Losses of certain B vitamins during cooking [1]

FOODS	RIBOFLAVIN Total content Before	After	Loss	NICOTINIC ACID Total content Before	After	Loss	PANTOTHENIC ACID Total content Before	After	Loss
	mg.	*mg.*	*%*	*mg.*	*mg.*	*%*	*mg.*	*mg.*	*%*
Meats									
1.* Beef round	88	69	22	1800	1700	5.5	190	130	32
2. Beef liver	1100	1300		4700	5200	0	2200	1900	14
3. Beef liver	2400	2300	4.2	14000	14000	0	8600	7400	14
4. Beef heart	1700	1700	0	15000	13000	13	3400	1900	44
5. Beef heart	890	950	0	8700	7400	15	2000	1400	30
6. Pork loin	130	87	33	1500	410	72	280	200	29
7. Pork loin	100	75	17	2300	1500	35	260	190	27
8. Pork loin	100	72	28	6200	4200	32	320	170	37
9. Bacon	43	51					110	170	
10. Bacon	69	63	8.7	920	940		220	110	23
11. Ham	140	110	21	1800	1700	5.5	180	170	
12. Ham	150	125	17	1900	2200	13	390	220	0
13. Veal chop	59	59	0	3000	2600	8.5	110	390	0
14. Veal chop	380	400	0	1200	1100	19		94	14
15. Lamb leg	1700	1700	0	52000	42000	0	4100	3600	12
16. Mutton shoulder	330	330	0	5300	5300	11	570	570	0
17. Chicken leg	110	330	0	1800	1600	18	320	250	22
18. Chicken leg	110	160		2290	1800	3.4	360	240	33
19. Chicken breast	40	40	5.9	3300	2800		180	160	
20. Chicken breast		160		3300	3400	7.0	730	740	5.9
21. Salmon	160	160	0	7100	6600	2.1	140	140	0
22. Halibut	110	86	22	9400	9200	5.6	9.5	9.5	0
23. Halibut	2.2	2.2	0	710	670				
Vegetables									
24. Beets	51	42	18	740	240	54	170	130	24
25. Cooking water	230	10		2300	570	18	420	9	18
26. Beets	86	230	0	240	800	61	56	260	36
27. Beet tops	57	74	14	210	230	4.2	180	36	28
28. Cabbage		48	16	210	170	19		130	
29. Carrots									
30. Cauliflower	150	130	13	780	610	22	970	970	0
31. Cauliflower	120	100	17	530	550		1000	940	6
32. Onions	50	37	26						
33. Potatoes	95	86	9.4	1600	1100	3.1			
34. Cooking water	92	46			100	0.6			
35. Potatoes		52	43	2600	1900	27			
36. Cooking water		40			700				
37. Lima beans	6.6	6.8	0	49	57		42	31	26
38. Okra	32	39	0	210	210	0	69	81	0
39. Rice	16	18	0	170	220		110	110	0
40. Sauerkraut	2.0	1.9	5.0	8.5	8.0	5.9	2.7	2.7	0
41. Spinach	220	160	27	510	430	16	160	150	6.3
42. Split peas	48	93	13						
43. Sweet potatoes	910	790	13	9980	9500	4.8			
44. Turnips	48	43	11	1100	660	0	160	94	41
45. Cooking water		8			440	0		54	
46. Turnips				15	16	0			7.5
Fruit									
47. Apples	580	430	25	67	50	25	1400	1500	0
Eggs and milk									
48. Eggs	655	341	48						
49. Eggs	205	160	22						
50. Eggs	360	380	0						
51. Eggs	360	380	0						
52. Milk	166	87	48						
53. Milk	166	130	22						

[1] The amounts of respective vitamins given in this table are those found in the number of grams of food analysed shown in the last two columns of table 1.

TABLE 2

Losses of certain B vitamins during cooking [1]

FOODS	BIOTIN Total content Before	After	Loss	INOSITOL Total content Before	After	Loss	FOLIC ACID Total content Before	After	Loss
	mg.	*mg.*	*%*	*mg.*	*mg.*	*%*	*mg.*	*mg.*	*%*
1.* Beef round	0.88	0.83	63	18	16	11	42	8.7	94
2. Beef liver		18	60	22	15	32	130	110	79 [a]
3. Beef liver	45		56	420	420	0	540	630	15
4. Beef heart			69	290	330	0	11	3.6	0
5. Beef heart	25	1.5	14	18	13	28	160	12	67
6. Pork loin	4.9	1.9	64	20	13	35	26	8.0	92
7. Pork loin	3.2	0.62	66	25	20	58	61	3.2	95
8. Pork loin	1.7	0.47	33	18	7.5	14	57	5.4	91
9. Bacon	1.4	2.0	18	14	12		25	1.9	87
10. Bacon	3.0	1.4	44	17	18	0	35	2.6	94
11. Ham	1.7	1.8	25	34	27	21	31	3.9	58
12. Ham	3.3	1.8	34	15	16	0	70	4.2	66
13. Veal chop	2.4	0.30	41	54	32	39	69	29	88
14. Veal chop	0.59	2.0	0	400	310	24	160	55	69
15. Lamb leg	3.4	15	33	68	77		800	100	64
16. Mutton shoulder	15	2.4	72	26	22	15	100	31	
17. Chicken leg	3.6	1.3	35	10	24	0	61	22	
18. Chicken leg	4.6	3.7	20	21	14	33			67
19. Chicken breast	5.7	1.4	43	19	19	25	63	21	62
20. Chicken breast	2.0	0.84	24	24	19	0	98	37	46
21. Salmon	1.5	4.5	30	.7	.7		67	36	74
22. Halibut	5.9	8.4	3				5.0	1.3	
23. Halibut	0.33	0.32							
24. Beets							120	7	94
25. Cooking water							170	5	90 [a]
26. Beets				80	43	46	84	50	71
27. Beet tops		1.1	9.1	8.3	3.9	54	65	23	74
28. Cabbage	1.1	1.0	0	95	62	35	48	5	92
29. Carrots	1.1	2.8		24	24	0	190	1.2	97
30. Cauliflower	2.4			96	93	3.1	110	23	88
31. Cauliflower		20	13	110	60	46		34	69
32. Onions	23	10	28				190	14	93
33. Potatoes	14							13	86 [a]
34. Cooking water							150	6	96
35. Potatoes								4	93 [a]
36. Cooking water									91
37. Lima beans	0.49	0.37	24	8.4	3.3	61	17	1.6	91
38. Okra	1.7	1.8	0	16	16	0	16	4.5	72
39. Rice	0.39	0.48	0	7.9	3.1	61	26	2.1	92
40. Sauerkraut	0.095	0.11	14	1.5	1.2	20	0.93	0.93	0
41. Spinach	5.9	5.1	21	27	20	26	170	27	84
42. Split peas	9.2	7.3		160	7.8	95			
43. Sweet potatoes							130	6	95 [a]
44. Turnips	0.46	0.51	0	10	11	0	11	8	89 [a]
45. Cooking water								1.5	86
47. Apples	4.3	4.5	0	19	20	0	110	38	65
							110	76	31

[1] The numbers in this column correspond to those in the first column of table 1. Cooking waters assayed separately and included with food samples.

Riboflavin

As has been pointed out previously (Williams and Cheldelin, '42), riboflavin losses during cooking tend to be greatest in the presence of light. Large losses, up to 48%, were incurred in the cooking of eggs, milk and pork chops.

Riboflavin losses sustained in the absence of light are, on the other hand, remarkably small. Steaming of meats or vegetables results, for the most part, in small or negligible losses of this vitamin. Similar observations have been made for meats by McIntire and coworkers ('43). The thermostability of riboflavin is indicated by the very slight destruction in baking sweet potatoes and roasting lamb. The contrast between losses in the presence and absence of light is especially marked for halibut, eggs and milk.

The opacity of many foods tends to prevent excessive destruction of riboflavin even in the light. Bacon, for example, fried for a relatively short time, retains essentially all of its riboflavin.

The apparent gains in riboflavin found with some samples are probably not significant. Substantial gains (greater than 10%) are often recorded with materials of relatively low potency where the accuracy of the assay method is poorest. In any case the assay method is probably accurate only to $\pm$ 10%. This qualification applies not only to riboflavin assays but to the others as well.

Nicotinic acid

Nicotinic acid is known to be quite stable to heat. Losses in cooking would therefore be expected to be slight. This is true for most of the samples listed in table 2, with the exception of pork chops, eggs and beets.

The small losses observed for meats are markedly lower than those reported by Waisman and Elvehjem ('41) and Dann and Handler ('42), using chemical methods of assay, although McIntire, et al. ('43) observed only slight losses in cooking of pork when microbiological assays were used. Since the results were obtained for different samples the reason for the

discrepancies is not apparent. It is possible that chemical changes in the antipellagric factor which accompany heating may not be reflected in the microbiological assay.

Pantothenic acid

Pantothenic acid losses are seen from table 2, to be moderate to slight among vegetables and eggs, as well as among such meats as bacon, ham, mutton and fish which are here subjected to relatively mild heat treatment. Beef heart and beets are exceptions. Frying of beef or pork for longer periods of time results in loss of about one third of the vitamin present. The results with meats are in general agreement with the data of Waisman and Elvehjem ('41), although the present losses are somewhat smaller for pork.

Biotin

Biotin losses among vegetables are moderate, ranging from 28% in steamed cauliflower to no loss in beets, cabbage, okra, rice, sauerkraut, and turnips. There is likewise no loss of biotin in frying eggs.

Losses in biotin among meats are generally higher, reaching 72% for fried chicken leg. Severity of heat treatment does not seem to be directly responsible for increased losses, since beef heart loses about 60% by steaming 30 minutes, whereas roasting of lamb at 150° C. for 2 hours results in practically no loss of biotin.

Inositol

Inositol losses among meats are quite variable, but are moderate in most cases. In general smaller losses are observed among samples which have been subjected to relatively mild treatment such as steaming (mutton, heart, and halibut). The greatest loss (58%) is found for one sample of fried bacon.

In contrast to most of the other B vitamins, inositol losses in cooking of vegetables are often much greater than those encountered for meats. Losses are especially high among the

legumes, with split peas losing 95% of their inositol content. In view of the stability of inositol toward heat, this is surprising especially since the severity of heat treatment of the vegetables tested was much less than that for most of the meats. It seems likely, therefore, that the losses observed may be due to "binding" of the vitamins in various tissue combinations rather than to thermal destruction.

Folic acid

Of all the vitamins studied, folic acid showed the greatest loss due to cooking. With the exception of liver and sauerkraut, which retain the bulk of their folic acid content, losses among meats range from 46% in halibut to 95% in pork chops; losses in vegetables range from 69% in cauliflower to 97% in carrots.

The reasons for such large losses are not known. Although the vitamin is unstable to light, it is not readily destroyed by autoclaving in the dark in the pH range common to most foods. It seems likely that folic acid may become "bound" in tissues during cooking. This may be investigated further at a later time.

SUMMARY

Cooking losses as determined with microorganisms, have been determined in thirty foods for riboflavin, nicotinic acid, pantothenic acid, biotin, inositol and folic acid. These are summarized below.

VITAMIN	DEGREE OF LOSS DURING COOKING
Riboflavin —	Destroyed in variable amounts in presence of light; negligible losses in the dark.
Nicotinic acid —	Generally slight.
Pantothenic acid —	Moderate to slight in vegetables; somewhat larger (up to one third) in meats.
Biotin —	Moderate to negligible in vegetables; quite high in meats (up to 72%).
Inositol —	Generally moderate among meats, with steamed samples only slight; often very great (up to 95%) in vegetables, especially in legumes.
Folic acid —	Very great for most foods.

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STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM IN THE CHICK

II. RELATIVE ANTIRACHITIC EFFECTIVENESS OF VITAMINS D_2 AND D_3 AND DIHYDROTACHYSTEROL ADMINISTERED PARENTERALLY [1]

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In a previous publication McChesney ('43b) reported a detailed study of the effects of vitamins D_2 and D_3 and dihydrotachysterol on the calcium and phosphorus metabolism of the chick. The various preparations were administered orally in corn oil, or intramuscularly in either propylene glycol or corn oil. Since the number of chicks used in the study was limited, and the vitamin dosages were either of the same unit value (as determined in the albino rat), or were intended to be antirachitically equivalent for the chick, too few points on the curve relating dose and response were established to permit anything better than a rough estimate of the relative effectiveness of the different preparations by the various routes of administration. The data obtained from these experiments were of sufficient interest, however, to warrant further studies of the three preparations administered by several parenteral routes. Under such conditions, differences in response due to failure of absorption from, or destruction in, the digestive tract may be entirely eliminated from consideration.

The objective of the study as a whole has been to secure information which would serve to explain the phenomenon

[1] Presented before the Division of Biological Chemistry of the American Chemical Society at Detroit, April 13, 1943.

first reported by Hess and Supplee ('30); namely, that vitamin D_2 is considerably less effective on a unit basis in the chick than natural vitamin D. In this paper an attempt has been made to establish so far as possible definite ratios of antirachitic effectiveness for the three preparations, administered either in propylene glycol or in oil, and by several parenteral routes. The ratios obtained from administration of the vitamin supplements in propylene glycol should, a priori, be of particular interest since this vehicle is readily absorbed in the tissues and does not remain in situ for long periods of time as does oil. In addition, propylene glycol may be injected intravenously. This affords an excellent means of transporting the vitamin to its site of utilization without passing it through the digestive tract or other tissues. Utilization should be as complete by this route as can be accomplished by any route of administration.

<div align="center">EXPERIMENTAL</div>

Male White Leghorn chicks were received in the laboratory on the second day of life. They were immediately offered water and the vitamin D-free diet previously described (Remp and Marshall, '38). From this point forward, with the exception of the vitamin supplementation, they were managed as if a vitamin D assay were being conducted according to the procedure outlined by Massengale and Bills ('36). The birds were divided into groups of 10 to 12 on the fourteenth day of life, the groups having equal average weights. The vitamin supplements were administered on the seventeenth, twenty-fourth, thirty-first, and thirty-eighth days of life; each chick received on these days an injection of 0.1 ml. into each breast muscle (total of 0.2 ml. per week) except that the chicks which received the intravenous or intraperitoneal injections received only 0.1 ml. The intravenous injections were made into the wing or leg vein.[2] On the forty-fifth day of life all the surviving chickens were sacrificed and both tibiae were removed.

[2] The author is indebted to Mr. Kimball D. Sprague for making these injections.

The bones were prepared for ashing in the manner recommended by Massengale and Bills. The data to be reported comprise three separate experiments, all conducted according to this general plan.

Experiment 1

This experiment was designed to compare the antirachitic effectiveness of the three preparations by the intramuscular route in propylene glycol and in oil. The relative effectiveness of propylene glycol solutions of vitamin D_3, administered intramuscularly, intravenously, and intraperitoneally was also studied. The pooled left tibiae obtained from each group at the end of the experiment were ashed. In certain of the more critical groups the right tibiae were also ashed individually in order that the probable error of the mean could be calculated. The data are given in table 1.

Experiment 2

The results of experiment 1 indicated that vitamin D_3 in propylene glycol is utilized to the same extent whether administered by the intramuscular or intravenous routes. These routes may therefore be considered, for practical purposes, to permit complete utilization of the vitamin, since the intramuscular injections give results which are superior to either the oral (McChesney, '43b) or the intraperitoneal routes (vide infra). These considerations made it of interest to investigate also the relative utilization of vitamin D_2 and dihydrotachysterol when administered by the intramuscular and intravenous routes in propylene glycol. Furthermore, additional data on the antirachitic effectiveness of these two preparations when given by the intramuscular route was desirable since the doses given in experiment 1 did not cover a sufficiently wide range.

This experiment was conducted in exactly the same manner as the preceding one except that the surviving chicks were

TABLE 1

Relative effectiveness of vitamins D₂, D₃, and dihydrotachysterol administered parenterally.

GROUP NO.	VEHICLE	TYPE OF INJECTION	PREPARATION	U.S.P. UNITS PER WEEK	PER-CENT WEIGHT GAIN [2]	PER-CENT MORTALITY	AVERAGE PERCENT BONE ASH Left tibiae [3]	Right tibiae [4]	EFFECTIVE UNITS PER WEEK [6]
1	Propylene glycol	Intramuscular	Vitamin D₂	25	99	33.3	30.39	30.22 ± 0.45 [5]	. .
2	Propylene glycol	Intramuscular	Vitamin D₂	50	127	8.3	33.20	32.84 ± 0.30	1.6
3	Propylene glycol	Intramuscular	Vitamin D₂	100	189	8.3	39.10	38.20 ± 0.61	7.2
4	Propylene glycol	Intramuscular	Vitamin D₃	5	168	0	36.21	37.14 ± 0.32	5 ⎱ Reference groups
5	Propylene glycol	Intramuscular	Vitamin D₃	10	185	0	42.75	42.03 ± 0.61	10
6	Propylene glycol	Intramuscular	Vitamin D₃	20	200	0	45.20	45.38 ± 0.26	20
7	Propylene glycol	Intramuscular	Dihydrotachysterol	1	100	0	33.33	33.94 ± 0.60	1.7
8	Propylene glycol	Intramuscular	Dihydrotachysterol	2	110	0	35.00	34.13 ± 0.31	3.6
9	Propylene glycol	Intramuscular	Dihydrotachysterol	4	152	0	35.32	35.45 ± 0.47	4.0
10	Propylene glycol	Intraperitoneal	Vitamin D₃	10	212	0	38.83		7.0
11	Propylene glycol	Intravenous	Vitamin D₁	10	217	8.3	42.20		9.6
12	Corn oil	Intramuscular	Vitamin D₂	500	127	25.	32.09	32.90 ± 0.55	0.2
13	Corn oil	Intramuscular	Vitamin D₂	1000	134	16.6	34.37	35.12 ± 0.48	2.9
14	Corn oil	Intramuscular	Vitamin D₃	100	212	0	40.27		8.1
15	Corn oil	Intramuscular	Vitamin D₃	200	195	0	46.19		23 (Approx.)
16	Corn oil	Intramuscular	Dihydrotachysterol	20 [1]	151	8.3	33.82		2.2
17	Corn oil	Intramuscular	Dihydrotachysterol	40 [1]	142	0	36.74		5.4
18	None	. . . .	None	0	127	8.3	31.91	32.20 ± 0.27	0

[1] One ml. of the commercial 0.5% solution assays 110 to 120 U.S.P. units in the albino rat, and contains about 1.25 mg. of the active principle.

[2] Based on the weights of the 45th day of life as compared to those of the 17th day of life.

[3] Ashed in groups.

[4] Ashed individually.

[5] Probable error of the mean.

[6] Calculated as vitamin D₃; based on the composite group ash values obtained from intramuscular administration in propylene glycol. (Groups 4, 5, 6, and 18).

sacrificed on the forty-third day of life instead of the forty-fifth. This change was considered advisable because in certain groups the birds were dying rather rapidly, and in others marked leg weakness indicated a severe grade of rickets, suggesting that the degree of depletion was greater than in the other experiments.

Individual bone ash determinations were made on the left tibiae, but the results in terms of vitamin D potencies are based on the composite of the groups (total weight of bone ash for the group divided by total weight of moisture and fat-free bones times 100), since this procedure was used in evaluating the results of experiment 1. This has the effect of placing a greater weight on the ash values of the larger birds. The results are given in table 2.

Experiment 3

The amounts of vitamin D_2 administered in oil in experiment 1 were insufficient to permit more than a slight degree of calcification. Therefore the effects of vitamin D_2 were investigated over a much wider dosage range in order to establish a more definite ratio between the requirements for vitamin D_2 in oil when given by the intramuscular and oral routes. Doses ranging from 500 to 16,000 units of D_2 per week were given intramuscularly in 0.2 ml. of corn oil, and their effects were compared with that of 1000 units per week orally (as a single dose) in 0.2 ml. of the same vehicle. The experimental procedures were the same as in experiments 1 and 2. Careful mortality records were kept, since the data reported in the earlier publication indicated greater mortality among chicks receiving 96 units per week of vitamin D_2 intramuscularly in oil than among negative control chicks injected with corn oil only. The symptoms observed suggested that this vitamin supplement aggravated the rickets rather than alleviated it. The results of this experiment are given in table 3.

TABLE 2

Utilization of vitamin D_2 and dihydrotachysterol when administered parenterally in propylene glycol.

GROUP	PREPARATION	ROUTE OF ADMINISTRATION	U.S.P UNITS PER WEEK	AVERAGE PERCENT WEIGHT GAIN[1]	PERCENT MORTALITY	PERCENT BONE ASH Average	PERCENT BONE ASH Composite	UNITS OF VITAMIN D₂ (CALCULATED FROM COMPOSITE BONE ASH)	
1	Vitamin D₃	Intramuscular	60	109	55	31.43 ± 0.91[3]	31.64	60	
2	Vitamin D₃	Intramuscular	125	126	0	35.11 ± 0.51	35.50	125	} Reference groups
3	Vitamin D₃	Intramuscular	250	195	0	37.26 ± 0.80	37.74	250	
4	Vitamin D₃	Intravenous	125	124	36[2]	36.23 ± 0.85	36.40	175	
5	Dihydrotachysterol	Intramuscular	4	116	18	32.49 ± 0.48	32.70	80	
6	Dihydrotachysterol	Intramuscular	7	134	0	36.12 ± 0.49	36.98	215	
7	Dihydrotachysterol	Intramuscular	10	176	9	38.54 ± 0.92	39.24	300 (Approx.)	
8	Dihydrotachysterol	Intravenous	7	104	27[2]	34.76 ± 0.45	34.90	130	
9	Vitamin D₃	Intramuscular	10	189	0	36.98 ± 0.34	36.65	190	
10	Propylene glycol	Intramuscular	0	44	27	29.10 ± 0.53	28.62	0	

[1] All chicks were sacrificed on the 43rd day of life. Percent gain refers to the average gain of the group between the 17th and 43rd days of life.

[2] Several chicks in these groups were killed accidentally while the injections were being made; they did not die as a result of vitamin deficiency.

[3] Probable error of the mean.

TABLE 3

Relative antirachitic effectiveness of vitamin D_2 given to chicks intramuscularly and orally in corn oil.

GROUP	ROUTE OF ADMINISTRATION	U.S.P UNITS PER WEEK	AVERAGE PERCENT WEIGHT GAIN [1]	PERCENT MORTALITY	AVERAGE LENGTH OF LIFE, DAYS [1]	PERCENT BONE ASH	
						Extreme range	Average [2]
1	Intramuscular	0	63	40	43.0	28.1–36.9	32.61 ± 0.92 [3]
2	Intramuscular	500	46	50	40.8	27.9–33.9	30.76 ± 0.94
3	Intramuscular	1000	115	30	42.1	32.8–40.8	35.30 ± 0.80
4	Intramuscular	2000	140	20	41.2	29.3–46.5	36.70 ± 1.39
5	Intramuscular	4000	108	20	41.1	30.2–45.7	38.04 ± 1.56
6	Intramuscular	8000	163	0	45.0	31.6–46.4	38.28 ± 1.26
7	Intramuscular	16000	235	10	43.5	34.9–47.1	43.07 ± 0.92
8	Oral	1000	169	0	45.0	35.7–42.1	38.56 ± 0.53

[1] All chicks were sacrificed on the 45th day of life.
[2] Ashed individually.
[3] Probable error of the mean.

DISCUSSION

While intramuscular and intravenous injections of vitamin D_3 in propylene glycol are equally effective, this may not be true of vitamin D_2 or of dihydrotachysterol. The data indicate that utilization of vitamin D_2 following intravenous injection was apparently 40% superior to that noted following intramuscular injections of the same supplement. However, when the probable errors of the means are taken into account, the difference between these groups (table 2, groups 2 and 4) is not statistically significant. Dihydrotachysterol appears to be about 40% less effective by the intravenous route than by the intramuscular route and in this case the differences noted are statistically significant, (table 2, groups 6 and 8). In both cases, however, the differences do not sufficiently exceed the experimental error to justify extensive speculation as to their cause unless they can be confirmed. In the one case in which intraperitoneal injections were given (table 1, group 10) the results show that there is probably some inactivation before the material reaches the blood stream, since the calcification was considerably inferior to that given by the same unit dose administered intramuscularly.

Utilization following intramuscular injections of vitamins D_2 and D_3 in propylene glycol is considerably superior to that noted after oral administration in oil. The first paper of this series showed that under the test conditions used the oral administration of 21 units of vitamin D_3 per week in oil gave a bone ash value of 39.6%, while the intramuscular injection of 20 units per week in propylene glycol gave a bone ash value of 45.6%. This difference clearly indicates that the utilization of the vitamin after intramuscular injection is two or two and one-half times superior to that obtained by the oral route (see data of Massengale and Bills, op. cit., p. 432).[3]

[3] These authors found that it required 6 units of natural vitamin D per 100 gm. of diet to produce a bone ash value of 39.6%, and 15.5 units per 100 gm. to produce a bone ash value of 45.6%. This indicates that the intramuscular dosage is about 1.5 times more effective than the oral. In this connection, our unpublished laboratory data on the albino rat show that vitamin D_3 in propylene glycol is about 50% more effective when injected intramuscularly than when given orally.

Although dihydrotachysterol is about 300% more effective per U.S.P. unit than vitamin D_3 when they are given orally to the chick, it is at the most 60% more effective (probably less) when they are given intramuscularly in either propylene glycol or oil. The most logical interpretation of this phenomenon is that when administered by way of the digestive tract the dihydrotachysterol is converted to a substance of considerably greater antirachitic value.

Vitamin D_3, administered intramuscularly in oil, is about one-fourth as effective in promoting calcification as when given orally, and about one-tenth as effective as when given intramuscularly in propylene glycol. Of vitamin D_2 and dihydrotachysterol, injected intramuscularly in oil, about one-ninth and one-twelfth, respectively, appear to become effective within the experimental period as compared to oral dosages. In the latter case, for reasons pointed out above, the fraction assigned is probably meaningless. The responses to vitamin D_2 administered intramuscularly in oil were very erratic, indicating large individual differences in absorption.

The peculiar phenomenon noted earlier, namely, that when doses of vitamin D_2 (equal in magnitude to about 10% of the curative dose) are given intramuscularly, they appear to exert an anticalcifying effect, has again been observed (table 1, group 1; table 3, group 2). Further study will be required to determine the significance of this observation.

In order to summarize in a convenient form the data obtained in these experiments, a table of the relative effectiveness of the different preparations administered by the several routes has been drawn up. It must be kept in mind that the preparation of such a table involves the carrying of results of one experiment over into another; this can be done exactly only if the negative controls give comparable results and certain positive responses are established as reference points in each experiment. Furthermore, quantitative comparisons which apply at the bone ash level selected would not necessarily apply at higher or lower levels. Because of the nature of the data available, all quantities have been evaluated in terms of those dosages required to produce a final bone

TABLE 4

Estimated requirements of the chick for three activated sterols in different vehicles and by several routes of administration.

PREPARATION	VEHICLE	ROUTE OF ADMINISTRATION	U.S.P. UNITS REQUIRED PER WEEK [1]
Vitamin D₃	Propylene glycol	Intramuscular	9 [2]
	Propylene glycol	Intravenous	9 [3]
	Propylene glycol	Intraperitoneal	13 [4]
	Corn oil	Intramuscular	90 [5]
	Corn oil	Oral	24 [6]
Vitamin D₂	Corn oil	Oral	850 [6]
	Corn oil	Intramuscular	8,000 [7]
	Propylene glycol	Intramuscular	150 [8]
	Propylene glycol	Intravenous	110 [9]
Dihydrotachysterol	Propylene glycol	Intramuscular	6 [10]
	Propylene glycol	Intravenous	10 [11]
	Corn oil	Intramuscular	75 [12]
	Corn oil	Oral	6 [6]

[1] To produce a standard response of 41–42% bone ash (average) under the experimental conditions, the negative controls at the same time having a bone ash of 31–32%.

[2] The table was compiled in the following manner. The values of the reference groups in table 1 (groups 4, 5, 6, and 18) were plotted on graph paper. From the graph it was determined that 9 units of vitamin D₃ administered intramuscularly in propylene glycol would have given the ''standard response'' (in this case, 41.9% bone ash since the negative controls gave 31.9% ash.) The remainder of the table is based on this one figure. Since all of the responses in table 1 were evaluated in terms of ''effective units,'' using the response to vitamin D₃ administered intramuscularly in propylene glycol as a basis, a table of equivalences is readily computed as given in detail below. The nearest whole or round number is used for obvious reasons.

[3] From table 1, groups 5 and 11; i.e., $9 \times \frac{10}{9.6} = 9$.

[4] From table 1, groups 5 and 10; i.e., $9 \times \frac{10}{7} = 13$.

[5] From table 1, group 5 and the average of groups 14 and 15 i.e., $9 \times \frac{100}{10} = 90$.

[6] From data presented in the first paper of this series.

[7] From table 3, groups 6, 7 and 8; i.e., the intramuscular dose must be 9–10 times the oral dose in order to have an equivalent antirachitic effect; $9 \times 850 = 8000$ approximately.

[8] From table 1, group 3 and table 2, group 9, which place the required dose of vitamin D₂ at from 14 to 19 times the dose of D₃.

[9] From table 2, groups 2 and 4, assuming the value obtained in footnote 8 to be correct; i.e., $150 \times \frac{125}{175} = 110$.

[10] From table 2, groups 7 and 9; i.e., (the reference value of) $9 \times \frac{190}{300} = 6$ approx.

[11] From table 2, groups 6 and 8; i.e., (from footnote 10) $6 \times \frac{215}{130} = 10$.

[12] From table 1, groups 16 and 17; i.e., (the reference value of) $9 \times \frac{20}{2.2} = 82$ or $9 \times \frac{40}{5.4} = 67$.

ash value (when the bones are ashed in groups) of 41–42%. The table has been based on Experiment 1, with the application of ratios of effectiveness derived from the other experiments where necessary to complete the table. In view of these considerations no claim is made as to the reproducibility of the numerical values given, but it is expected that the ratios of effectiveness derived from them would generally be reproducible within ± 25%. The data are given in table 4.

SUMMARY

The relative effectiveness of vitamin D_2 and D_3 and dihydrotachysterol when administered parenterally to the chick in propylene glycol and corn oil has been studied. Rather definite requirements have been established for the different preparations by the several routes of administration and are presented in the form of a table. Although the weekly oral requirements (as defined herein) of the three preparations administered in oil are: vitamin D_3, 24 units; vitamin D_2, 850 units; and dihydrotachysterol, 6 units (i.e., in the proportions of 1:35:0.25), the intramuscular requirements in propylene glycol (in the same order) are 9,150, and 6 units (i.e., in the proportions of 1:16:0.7) and the intravenous requirements (in the same order) are 9,110, and 10 units (i.e., in the proportions of 1:12:1.1). Thus although dihydrotachysterol is 300% more effective than vitamin D_3 by the oral route, it averages only about 20% more effective by the parenteral routes. The data on relative oral and parenteral utilization of vitamins D_2 and D_3 indicate that the latter is somewhat better absorbed from the digestive tract.

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THE EFFECT OF THIAMINE, RIBOFLAVIN OR PYRIDOXINE DEFICIENCY ON THE INTESTINAL ABSORPTION OF GALACTOSE IN THE RAT[1]

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Previous reports from this laboratory (Free and Leonards, '42; Leonards and Free, '43) have indicated that the rate of intestinal absorption of galactose of pair-fed litter-mate control rats was 35% more than that of animals deficient in the vitamin B complex and 15% more than that of animals deficient in pantothenic acid. The present report describes studies of the effect of deficiency of other members of the vitamin B complex on intestinal absorption.

METHODS

Three groups of young albino and piebald rats of both sexes were placed on a diet consisting of casein, sugar, starch, cottonseed oil, cod liver oil and choline chloride identical with that previously used in this type of study (Leonards and Free, '43). The daily vitamin B supplements per rat were thiamine 40 µg.; riboflavin 25 µg.; pyridoxine 20 µg.; and calcium pantothenate 100 µg.[2] By the omission of the proper

supplement one group of rats received a diet deficient in thiamine, another group in riboflavin and the third group in pyridoxine. Each experimental animal was "paired" with a litter-mate of the same sex which served as a control. The control animals received all of the above vitamin supplements. The food intake of the control member of the pair was regulated so that its weight was kept nearly the same as that of the experimental member.

The thiamine deficient rats were kept on the diet for 38 to 45 days at which time the animals showed deficiency symptoms similar to those observed in our previous study on vitamin B complex deficiency (Free and Leonards, '42). At this time the studies of intestinal absorption were carried out. Polyneuritis was not observed in any of the rats.

The animals deficient in riboflavin grew very slowly and ceased gaining weight after about 3 weeks. At this time vascularization of the cornea was observed in six of the twenty-six experimental animals. The studies of intestinal absorption were carried out after 38 to 48 days at which time the animals had started to lose weight.

Measurements of the rate of intestinal absorption were carried out on the pyridoxine deficient rats after they had been on the diet for 40 days. The only deficiency symptom which could be observed was the failure to gain weight. The technics for the measurement of intestinal absorption and blood galactose level employed in this study were identical with those already described (Free and Leonards, '42). Exactly 1 hour after ingesting 6 gm. per kilogram of galactose the animals were sacrificed and the amounts of the sugar remaining in the stomach and intestines were determined separately. The galactose absorbed is the difference between the amount of sugar ingested and that found in the gastrointestinal tract. The coefficient of absorption is a value expressing the milligrams of galactose absorbed per 100 gm. of body weight per hour. The level of galactose in the blood was also determined at the time the animals were sacrificed and is used as an indirect measure of the rate of intestinal absorption of the sugar.

RESULTS AND DISCUSSION

Thiamine deficiency

The results of the absorption measurements on fourteen pairs of rats in which the experimental member of each pair was deprived of thiamine are shown in table 1. In all of the tables albino rats are designated by W and piebald animals by P. The coefficient of absorption is a value expressing the milligrams of galactose absorbed per 100 gm. of body weight per hour. The average coefficient of the thiamine deficient animals was 218 while the controls averaged 361 mg. per 100 gm. per hour. Although there is considerable variation in absorption coefficients among the individual animals, it is significant that in all but one of the fourteen pairs of rats that were studied the coefficient of the control member was greater than that of the thiamine deficient rat. It is also significant that the impairment of intestinal absorption in thiamine deficiency is even greater than the impairment which we have previously observed (Free and Leonards, '42) when rats were deficient in all the members of the vitamin B complex. In both the deficient and control animals a considerable percentage of the ingested galactose remained in the stomach, amounting to 41% in the deficient animals and 27% in the controls. However, the intestines of the deficient rats contained more galactose than the controls; and since this is the sugar available for absorption, gastric evacuation probably was not an important factor in causing the thiamine deficient animals to exhibit a decreased rate of intestinal absorption. The results of the present study do not indicate the mechanism by which the thiamine deficiency exerts its effect on intestinal absorption.

The blood galactose levels of the deficient rats 1 hour after the ingestion of the sugar averaged 427 mg. per 100 ml. while the controls averaged 566 mg. per 100 ml. In each of the thirteen pairs in which the absorption coefficient of the deficient member was less than that of the control, the same result prevailed for the blood galactose level. Thus it is again evident that the blood galactose level is an indirect measure of the

TABLE 1

The effect of thiamine deficiency on absorption coefficients and blood galactose levels.

RAT			DEFICIENT ANIMALS						CONTROL ANIMALS						DIFFERENCE (CONTROL MINUS DEFICIENT)	
			Body weight		Galactose				Body weight		Galactose					
Pair	Strain	Sex	Initial	Final	In stomach[1]	In intestines[1]	Absorption coefficient	In blood	Initial	Final	In stomach[1]	In intestines[1]	Absorption coefficient	In blood	Absorption coefficient	Blood galactose
			gm.	gm.	%	%	gm./hr	ml.	gm.	gm.	%	%	gm./hr	ml.		
							mg./100						*mg./100*			
1	P	M	33	35	53	19	168	447	33	31	24	16	360	500	+ 192	+ 53
2	P	M	32	31	62	34	24	187	33	35	17	11	432	680	+ 408	+ 493
3	P	M	51	44	37	17	276	:	51	60	36	8	336	:	+ 60	: :
4	P	M	51	49	41	20	216	:	58	54	44	14	252	:	+ 36	: :
5	P	M	28	26	40	15	270	557	40	41	24	12	384	620	+ 114	+ 63
6	P	M	38	33	32	31	222	474	41	43	26	14	360	648	+ 138	+ 174
7	P	M	40	33	28	18	324	630	34	40	42	12	276	465	− 48	− 165
8	P	M	37	29	28	27	270	594	36	44	25	14	366	660	+ 96	+ 66
9	P	F	33	30	45	24	186	201	30	37	1	10	534	575	+ 348	+ 374
10	P	F	38	37	38	25	222	460	36	50	24	18	348	562	+ 126	+ 102
11	P	F	36	32	50	29	126	451	39	46	38	14	288	540	+ 162	+ 89
12	P	F	31	46	70	16	84	290	28	38	46	13	246	379	+ 162	+ 89
13	W	F	38	37	29	12	354	268	43	49	13	11	456	589	+ 102	+ 321
14	W	M	40	40	28	20	312	558	35	42	21	9	420	577	+ 108	+ 19
Average			37	36	41	22	218	427	38	44	27	13	361	566	+ 143	+ 140

[1] Fraction of ingested sugar found in the organ.

rate of absorption of the sugar. However, the blood galactose levels of the deficient rats are somewhat higher than would be predicted from the absorption coefficients, a fact indicating that the rate of removal of absorbed galactose from the blood is somewhat slower than normal. This may be due to an impairment in either the metabolism or excretion of the sugar. These findings are similar to those which we have observed in pantothenic acid deficiency (Leonards and Free, '43) and are also in accord with the work of Williams and co-workers ('40), who found a decrease in the glucose tolerance during experimental thiamine deficiency in human subjects. Elsom and co-workers ('40) also noted a decrease in glucose tolerance in a human subject rendered deficient in the vitamin B complex. Lepkovsky, Wood and Evans ('30) found an impairment in glucose tolerance of thiamine deficient rats and Pachman ('31) showed that such rats exhibit a decreased glucose tolerance when the sugar is injected intravenously.

Riboflavin deficiency

The average absorption coefficient of the riboflavin deficient animals was 352 mg. per 100 gm. per hour whereas that of their paired controls averaged 367 mg. per 100 gm. per hour. This difference is well within the experimental error. The individual results are given in table 2. There were the expected variations in absorption coefficients among the individual animals. In fifteen of the twenty-six pairs the absorption coefficient of the control member exceeded that of the deficient companion while the reverse was the case in the other eleven pairs. There is little doubt that under the conditions of these experiments a deficiency of riboflavin was without effect on the rate of absorption of galactose. These observations do not support the hypothesis that riboflavin is intimately connected with intestinal absorptive function (Sciclounoff, F. and Alphonse, P., '41; Antognini, R., '41). Beams, Free and Glenn, '41, noted that two of four cases of rosacea keratitis which responded to riboflavin therapy gave indication of decreased absorption of galactose which improved after therapy when the signs of rosacea keratitis disappeared.

TABLE 2

The effect of riboflavin deficiency on absorption coefficients and blood galactose levels.

RAT			DEFICIENT ANIMALS						CONTROL ANIMALS						DIFFERENCE (CONTROL MINUS DEFICIENT)	
			Body weight		In stomach [1]	In intestines [1]	Galactose		Body weight		In stomach [1]	In intestines [1]	Galactose		Absorption coefficient	Blood galactose
Pair	Sex	Strain	Initial	Final			Absorption coefficient	In blood	Initial	Final			Absorption coefficient	In blood		
			gm.	gm	%	%	mg/hr gm/hr	mg/100 ml	gm.	gm	%	%	mg/hr gm/hr	mg/100 ml		
1	F	P	36	39	29	18	318	330	38	37	16	12	432		+ 114	..
2	F	P	29	25	20	21	354	598	26	28	42	17	246	460	— 108	— 138
3	F	P	35	32	6	16	468	760	36	40	18	13	414	601	— 54	— 159
4	F	P	29	32	22	16	372	506	40	35	51	11	228	423	— 144	— 83
5	M	P	37	43	14	13	438	642	37	33	30	14	336	507	— 102	— 135
6	M	P	25	27	32	6	372	598	22	29	15	8	462	657	+ 90	+ 59
7	M	P	30	33	24	22	324	492	30	35	17	54	174	425	— 150	+ 67
8	M	P	27	33	46	28	156	331	30	39	34	25	246	465	+ 90	+ 134
9	M	P	25	29	54	18	168	401	30	42	25	13	372	635	+ 204	+ 234
10	M	P	42	45	27	20	318	564	30	43	28	12	360	600	+ 42	+ 36
11	F	W	29	45	20	14	396	491	27	45	23	10	402	521	+ 6	+ 30
12	F	W	32	34	26	13	366	561	26	30	5	14	486	697	+ 120	+ 136
13	F	W	37	42	15	31	324	392	34	46	9	34	342	452	+ 18	+ 60
14	F	W	32	40	12	22	396	502	32	43	40	7	318	465	— 78	+ 37
15	F	W	89	77	24	15	366	631	87	77	24	12	384	735	+ 18	+ 104
16	F	W	95	90	37	10	318	640	75	88	32	9	354	618	+ 36	+ 22
17	F	W	26	30	2	15	495	611	30	36	11	10	474	548	— 24	+ 63
18	F	W	30	32	1	40	354	331	28	35	19	11	420	600	+ 66	+ 269
19	F	W	69	64	38	13	294	448	28	45	27	11	378	604	+ 84	+ 156
20	F	W	26	35	20	12	408	688	28	39	26	13	366	657	— 42	+ 31
21	M	W	40	40	27	18	330	572	42	34	15	2	498	657	+ 168	+ 85
22	M	W	32	41	15	14	426	600	44	44	23	13	384	577	— 42	— 23
23	M	W	31	46	11	13	456	732	30	40	8	9	498	593	+ 42	— 139
24	M	W	42	50	14	13	438	577	40	47	23	12	390	550	— 48	+ 27
25	M	W	23	29	16	23	366	607	27	36	34	18	288	532	— 78	+ 75
26	M	W	108	112	63	14	138	282	98	112	43	8	294	524	+ 156	+ 242
Average			40	44	24	18	352	543	38	44	24	14	367	564		

[1] Fraction of ingested sugar found in the organ.

Figures for blood galactose indicate an average value of 543 mg. per 100 ml. for the deficient animals and 564 mg. per 100 ml. for the controls. In about one-half of the pairs the blood galactose level of the control member was greater than that of the deficient member, the reverse being true in the other pairs. In general the blood galactose levels corresponded to the rate of intestinal absorption as measured directly by the absorption coefficients. In only two of the twenty-six pairs (nos. 16 and 23 in table 2) did the comparison of the blood galactose levels of deficient and control members not correspond with their absorption coefficients.

Pyridoxine deficiency

The individual results of the absorption measurements of thirteen pairs of rats in which the experimental member was deprived of pyridoxine for 40 days are given in table 3. The average absorption coefficient of the deficient rats was 304 mg. per 100 gm. per hour as compared with 340 mg. per 100 gm. per hour for the controls. The average blood galactose levels were respectively 550 mg. per 100 ml. and 597 mg. per 100 ml. for the deficient and control groups. Thus as evidenced by both direct measurement of the rate of intestinal absorption and by blood galactose levels there was a slight decrease in the rate of absorption of galactose in the deficient animals. The decrease amounted to about 10%. This may perhaps be significant since in nine of the thirteen pairs the rate of absorption was less in the deficient than in the control member of the pair. It is also interesting to note that the fraction of ingested galactose remaining in the stomachs of the deficient and control animals was identical, whereas the average amount in the intestines of the deficient rats was more than in the intestines of the control rats.

It is evident that of the members of the vitamin B complex that were studied, a deficiency in thiamine resulted in the greatest degree of impairment of intestinal absorption of galactose. This may be due in part to the fact that the thiamine deficient animals were in a more severe state of deficiency.

TABLE 3

The effect of pyridoxine deficiency on absorption coefficients and blood galactose levels.

RAT			DEFICIENT ANIMALS						CONTROL ANIMALS						DIFFERENCE (CONTROL MINUS DEFICIENT)	
			Body weight		Galactose				Body weight		Galactose				Absorption coefficient	Blood galactose
Pair	Strain	Sex	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood	Initial	Final	In stomach [1]	In intestines [1]	Absorption coefficient	In blood		
			gm.	gm.	%	%	mg/hr gm/hr	mg/100 ml.	gm.	gm.	%	%	mg/hr gm/hr	mg/100 ml.		
1	W	M	41	59	32	10	348	474	44	65	45	7	288	487	− 60	+ 13
2	W	M	44	63	39	13	288	612	40	65	43	10	282	604	− 6	− 8
3	W	M	43	60	48	23	174	418	41	60	10	11	474	781	+ 300	+ 363
4	W	M	46	68	39	10	306	621	46	67	48	10	252	514	− 54	− 107
5	W	M	45	55	22	11	402	728	34	58	36	9	330	576	− 72	− 152
6	W	M	43	61	33	10	342	576	43	61	29	8	378	697	+ 36	+ 121
7	W	M	41	60	32	19	294	563	41	66	34	6	360	577	+ 66	+ 14
8	W	M	41	57	25	21	324	460	41	61	27	12	366	612	+ 42	+ 152
9	W	M	38	60	30	25	270	415	32	53	32	8	360	585	+ 90	+ 170
10	W	M	41	51	39	15	276	585	40	52	42	10	288	581	+ 12	− 4
11	W	F	45	57	23	17	360	617	41	57	28	8	384	635	+ 24	+ 18
12	W	F	34	49	42	15	258	599	44	74	39	10	306	545	+ 48	− 54
13	W	F	35	49	34	14	312	465	31	49	27	14	354	565	+ 42	+ 100
Average			41	58	34	16	304	550	40	60	34	10	340	597		

[1] Fraction of ingested sugar found in the organ.

Under the conditions of these experiments, a deficiency of riboflavin or pyridoxine was not followed by any evidence of gross intestinal dysfunction. However, the absorption studies in these animals were made at a time when the animals began to lose weight but before the occurrence of any crisis or severe deficiency symptoms. Thus these findings do not lend themselves to a prediction of the effect of acute stages of riboflavin or pyridoxine deficiency on the intestinal absorption.

SUMMARY

Studies of the rate of intestinal absorption of galactose were carried out in rats deficient in thiamine, rats deficient in riboflavin and rats deficient in pyridoxine. Each deficient animal was paired with a litter-mate control which received adequate supplements of the missing vitamin.

The rate of intestinal absorption of galactose of pair-fed litter-mate controls averaged 66% more than that of the thiamine deficient animals, and 12% more than that of the pyridoxine deficient rats. Riboflavin deficiency had no effect on the rate of intestinal absorption of galactose in the rat.

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A STUDY OF NORMAL HUMAN REQUIREMENTS FOR ASCORBIC ACID AND CERTAIN OF ITS METABOLIC RELATIONSHIPS [1]

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The necessity for further research to determine specific nutrient needs for individuals was emphasized in the recommendations made to the President by the National Nutrition Conference for Defense ('41). Kruse ('42) discussed our need for determining optimal intakes of ascorbic acid since it is evident that a large proportion of the population show a past dietary regimen inadequate with respect to this nutrient. As recently as September, 1942, Gordon ('42) stated "the question of the daily human requirement of ascorbic acid must be considered still unsettled". Our need for more knowledge concerning optimal intake of food nutrients was recently summarized in Nutrition Reviews ('43) in the statement that a "good" diet must furnish an optimal quantity of all essential food factors. A number of investigators (Göthlin, '33, Hawley, Stephens and Anderson, '36, Widenbauer, '37, and Abbasy, Harris, Ray and Marrack, '35) who have sought to determine human requirements for ascorbic acid have obtained figures which are now recognized as the minimal intake necessary to prevent the manifestation of a

deficiency of this substance. It was also noted that several investigators (Gaehtgens and Werner, '37, Baumann and Rappolt, '37, Todhunter, Robbins and McIntosh, '42 and Crandon, Lund and Dill, '40) have used the term "at least" in connection with some standard daily requirement figures for ascorbic acid. Studies attempting to develop a means for determining optimal intakes of ascorbic acid are very few. The requirement figures for ascorbic acid occurring in the literature vary from 20 to 150 mg. per day, and from 0.4 mg. to 2.0 mg. per kilogram of body weight.

The principal factor contributing to our lack of knowledge concerning an optimal intake of ascorbic acid has been the disagreement among various laboratories as to the best method for determining human requirements. The use of the saturation test dose method for determining these requirements has met with success in the laboratories of several workers (Goldsmith and Ellinger, '39, Ludden and Wright, '40, Wright, Lilienfeld and McLenathan, '37, and Glazebrook and Thomson, '42). The main reason for variations in results obtained by this method seems to be due to the manner in which the test dose is administered. Several investigators have compared the validity of results obtained with oral versus intravenous administration of the test dose. Ludden and Wright ('40), Wright, Lilienfeld and McLenathan ('37), Faulkner and Taylor ('38) and Hawley and Stephens ('36) have compared the oral and intravenous technics as means of measuring absorption and utilization of a test dose. These investigators obtained more consistent results in any one individual when the test dose was administered intravenously. Their work gave evidence that, in response to a specific dose administered orally, great variations could be expected due to physiological differences in the rate of absorption and utilization.

Ludden and Wright ('40), Harris and Ray ('35), Levcowich and Batchelder ('42) and Hawley, Stephens and Anderson ('36) have determined that long time balance studies of

ascorbic acid are unnecessary for the investigation of normal requirements.

Since our knowledge as to the manner in which ascorbic acid functions is far from complete, there are no specific means at present by which optimal as contrasted to adequate intakes of ascorbic acid can be measured. The authors of this study proposed to determine an optimal utilization figure by measuring the blood concentration and urinary elimination of a relatively large number of apparently normal individuals in response to a test dose administered intravenously.

EXPERIMENTAL PROCEDURE

College students including both men and women and a few women staff members served as subjects for this study. The men were from the Department of Biochemistry and the women represented majors and instructors in the Department of Foods and Nutrition.

The subject to be tested was given a definite diet with an ascorbic acid content of 15 mg. for the males and 14 mg. for the females. For 24 hours prior to the administration of the test dose of ascorbic acid the subject was requested to continue his normal activities. The amount of water consumed throughout the diet period was not restricted. A "fasting" 24-hour urine sample was collected from the subjects upon this diet. A "fasting" sample of blood was taken before breakfast on the day of administration of the test dose. This sample was taken from the finger and was obtained in order to determine whether or not the subject being studied was to be classed in the normal or subnormal group as regards the fasting content of ascorbic acid in the body. In these studies, using the evidence of Wright, Lilienfeld and Mc-Lenathan ('37), Ludden and Wright ('40), and Thysell ('39), all subjects whose ascorbic acid content of the "fasting" plasma was 0.8 mg. per 100 ml. or above were classed as normal and those whose "fasting" plasma values were below this figure were classed as subnormal. (In the evaluation of the results obtained, these two classifications will be con-

sidered separately as well as combined, to determine the percentage of an apparently normal group of individuals who actually showed past nutritional inadequacy with respect to ascorbic acid, and to determine the requirements of individuals who gave proof of an adequate past dietary regimen.)

Although the original plans for this study called for the administration of the test dose intravenously, due to circumstances beyond our control, it was necessary in some cases to give the test dose by mouth. After the "fasting" blood sample was taken, the 500 mg. test dose of ascorbic acid was given, either orally or intravenously (500 mg. in 5 ml. physiological saline [2]). The diet for the 24 hours following administration of the test dose was exactly the same as that of the previous day. The response of the subject to the test dose was followed in the blood stream by measuring the ascorbic acid of the plasma at regular intervals until the absorption of the dose (either from the intestine or from the blood into the tissues) was indicated by a return of the plasma value to the fasting level. The response to the test dose was followed simultaneously in the urinary excretion values. The urine for the entire 24 hours following administration of the test dose was analyzed, at definite intervals, and as soon as possible after voiding. It was necessary to know the actual quantity of ascorbic acid of the test dose excreted in the 24 hours after administration in order to determine the quantity metabolized by the body during this period.

After determining the quantity of ascorbic acid excreted in the 24 hours following administration of this test dose, it was possible to determine the quantity retained by the particular individual being studied. To do this, the quantity of ascorbic acid present in the diet was taken into account, and the level of ascorbic acid in the "fasting" urine sample was taken into consideration. By subtracting the quantity of

ascorbic acid excreted by the individual in the test period from the quantity administered (both in the diet and in the test dose), a retention figure was obtained. By subtracting the quantity of ascorbic acid excreted by this individual under "fasting" conditions from this retention figure, a value was obtained which has been considered in this study the quantity of ascorbic acid metabolized (and therefore, probably required) by the subject in question.

In the course of this study, certain metabolic relationships of ascorbic acid were investigated. Hemoglobin values and the urinary output of citric acid were determined before and after the administration of the test dose of ascorbic acid. Observations were also made on the relation of the ascorbic acid requirement to the basal metabolic rate.

The methods described by the authors in a previous paper (Purinton and Schuck, '43) were used for the determination of ascorbic and citric acids.

For the determination of hemoglobin, the method of Breuer and Militizer ('38) was used, the only modification being the use of ammonium persulfate in place of potassium persulfate suggested by these workers.

The method used for determining the human requirement values for ascorbic acid was checked for validity by means of repeated tests on one person. Altogether, five determinations were completed on this individual. The determinations were made at different seasons, to offset the possibility of the influence of fresh fruits and vegetables in the past dietary history. The greatest variation noted in the different determinations for this individual was only 6.5 mg. per day. The fact that this variation was so small was interpreted to be a good indication of the applicability and validity of this method for the determination of human requirements. The plasma content of ascorbic acid for this subject under fasting conditions was at all times well above the standard normal value used in this study (0.8 mg. ascorbic acid per 100 ml. plasma).

RESULTS

Sixty-three apparently normal individuals, 52 women, aged 16 to 49 years, and 11 men, aged 20 to 25 years, were studied by means of the saturation test dose method for determining human requirements for ascorbic acid. Forty-six per cent of these had "fasting" plasma ascorbic acid levels of 0.8 mg. or more per 100 ml. of plasma. Citric acid excretion was determined for 42 of these subjects. The test dose of ascorbic acid was administered by injection to 47 subjects and by mouth to 16 subjects. The results are summarized in table 1.

TABLE 1

Ascorbic acid requirements [1] and citric acid excretion.

CATEGORY OF INTEREST	AGE IN YEARS AND SEX			
	15 – 20 women	20 – 25 women	20 – 25 men	25 – 50 women
No. subjects	8	29	11	15
Aver. "fasting" plasma asc. acid, mg./100 ml.	0.72	0.71	0.76	0.88
Aver. "fasting" urine asc. acid, mg./day	20.1	25.6	19.7	28.2
Asc. acid administered in test period (diet + test dose) mg.	514	514	515	514
Aver. urinary excretion in test period, mg./day	381.4	382.8	370.6	404.9
Aver. requirement, mg./day	112.5	105.6	124.7	80.9
Aver. requirement, mg./Kg.	1.95	1.86	1.71	1.50
Aver. citric acid excreted prior to test dose, gm./day	0.55	0.38	0.54	0.69
Aver. citric acid excreted after test dose, gm./day	0.80	0.66	0.90	1.24

[1] Results obtained from orally administered doses and with subjects showing very low "fasting" plasma levels accompanied by unusually high retentions were not used in computing average asc. acid requirements.

It is of interest to note that the younger women subjects showed a higher ascorbic acid requirement than the older ones. Also it was found that the average requirement figure for those with low "fasting" plasma ascorbic acid levels was higher than that for subjects with "fasting" plasma levels of 0.8 mg. or more per 100 ml. plasma.

Hemoglobin studies were made upon 19 of the subjects. It was found that 17 or 89.5% showed a definite correlation of hemoglobin in the blood with ascorbic acid content of the plasma. Only 2 individuals or 10.5% failed to show this correlation. In general, these individuals whose concentration of ascorbic acid in the fasting blood sample was above the figure used in this study as a normal fasting level (0.8 mg. per 100 ml. plasma) also had concentrations of hemoglobin above normal (15.6 gm.-Haden). Those subjects with low concentrations of ascorbic acid in the fasting sample were found to have low levels of hemoglobin. The incidence of low hemoglobin content of the blood was greater in the women subjects than in the men.

Of 16 subjects reporting a low basal metabolic rate, 68.8% had an ascorbic acid requirement below the average.

In table 2 are found figures on the relationship between ascorbic acid retention and citric acid excretion which appear

TABLE 2

Ascorbic acid retention and citric acid excretion.

	NO. OF SUBJECTS	TOTAL CITRIC ACID EXCRETED AFTER ADM. TEST DOSE ASC. ACID AVG GM /DAY	INCREASE IN CITRIC ACID OUTPUT AFTER ADM. TEST DOSE ASC. ACID AVG GM /DAY
Total	39		
Retention asc. acid below avg.[1]	22	0.93 (Range 0.41–2.30)	0.41 (Range 0.08–1.08)
Retention asc. acid above avg.	17	0.75 (Range 0.25–1.54)	0.35 (Range 0.05–0.94)

[1] The average retention figure for this group following administration of test dose of ascorbic acid was 138.8.

The differences were considered as having some meaning because they represent quantities greater than the total citric acid output of some of these subjects under fasting conditions.

to confirm the findings in animal studies reported in an earlier paper (Purinton and Schuck, '43). It was observed with our human subjects that lowered retention of ascorbic acid was accompanied by an increase in the total quantity of citric acid excreted. Conversely, high retention of ascorbic acid was accompanied by a lower excretion of citric acid than was noted when retention of ascorbic acid was low.

DISCUSSION

The daily human requirements for ascorbic acid, determined in this study, were found to be higher than most figures occurring in the literature.

Since slightly more than half of the subjects had "fasting" plasma ascorbic acid levels of less than 0.8 mg. per 100 ml. plasma, the high requirement figures may reflect in part the need for ascorbic acid for tissue saturation.

An optimal intake for the young adult appears to be in excess of 100 mg. per day. For adults between 25 and 50 years of age, less than 100 mg. per day appears to represent an optimal intake. This lowered requirement for older subjects is what might be expected since bodily requirements in general tend to decrease with maturity.

The orally administered test dose for determining human requirements for ascorbic acid by the saturation test dose method did not in this study give results comparable to the injected dose. In most cases the proportion of the oral test dose excreted during the 24 hour test period was small and retention figures averaged much higher than was the case with the injected test dose.

The findings of this study seem to indicate that requirements for ascorbic acid are influenced by certain factors, such as basal metabolic rate, and hemoglobin levels.

The relationship noted between ascorbic acid retention and citric acid excretion might be interpreted as signifying that citric acid plays a definite role in the metabolism of ascorbic acid, or that ascorbic acid functions in the metabolism of citric acid.

SUMMARY

1. The daily requirements for ascorbic acid of a group of 63 individuals, 52 women and 11 men, were determined by use of the saturation test dose method administered intravenously.

2. A requirement greater than 100 mg. daily was obtained for the subjects under 25 years of age. The requirement for a small number of older women between 25 and 50 years of age was well below 100 mg.

3. A correlation between the ascorbic acid of the plasma and the hemoglobin level was found in 17 out of 19 individuals.

4. The basal metabolic rate also appeared to be related to the ascorbic acid requirement, this requirement being less for subjects with low basal metabolism.

5. Confirmation of earlier findings with animal studies on the existence of a relation between ascorbic acid retention and citric acid excretion was obtained.

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THE NUTRITIVE VALUE OF PROTEIN

I. THE EFFECT OF PROCESSING ON OAT PROTEIN

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It is perhaps not well known that cereal grain is the largest single contributor of protein to the national dietary. At the White House Conference on Child Health and Protection ('32) it was reported that 36% of the protein in the national dietary was supplied by grains while only 26% was supplied by meat, 20% by dairy products and 18% by miscellaneous sources. These figures are probably not representative of the civilian dietary today, for it is evident that less animal protein is being consumed by the average civilian than in the pre-war period.[1]

Since animal proteins have long been considered superior in quality to the plant proteins the statement that "there is no need to include protein of an animal origin in the diet of man whatever the needs to be satisfied, provided that the choice of plant foods is not too much restricted" becomes significant (Cuthbertson, '40).

In view of the knowledge that cereals have such wide-spread use and when used freely with other plant proteins may supply protein for adequate human nutrition, it is pertinent to determine how best the cereal proteins can be used to replace unavailable animal protein. The efficacy with which this is done depends in part on the biological value of the cereal in the form in which it is offered for human consumption.

[1] In Jan. 1943, the estimated average per capita consumption of meat was 93.2% of the 1941 figure (Annal. Am. Acad. Political and Soc. Sci., Jan., 1943, p. 107).

Several investigators have shown that processing of cereals affects the nutritive value of the protein. Morgan ('31) has demonstrated that severe heat treatment adversely affects wheat protein. These observations have been substantiated by Murlin and associates ('38). So far as we are aware, nothing has been reported on the effect of cooking or processing on the nutritive value of oat protein. Since nutritionists (Wilder and Keyes, '42, Maynard, '42, Murlin et al., '38) have recognized the superior protein quality of oats, it is of interest to learn whether the superior quality of oat protein is carried through to the ultimate consumer. Accordingly, an investigation of the protein quality of the various oat products that have appeared on the market has been undertaken.

Oats are generally cooked in the home, but at least three and possibly four precooked oat products are on the market and each of them is prepared by a different cooking technique, so they offered an ideal source of material for this study. These products are:

(1) Drum-dried oatmeal commonly found on the market as a precooked baby food. The product used in these tests was made from oat flour batter kept at the boiling point of water for 15 minutes and then dried in thin flakes in 15 seconds on a drum-drier held at 130° C.

(2) Oven expanded oat cereal [2] made by cooking oat flour in dough form in a jacketed tube under pressure of 100 lb. for 1 to 2 minutes, forming into globules and expanding the globules in approximately 1 to 2 minutes in an oven held at 200° C.

(3) A ready-to-eat cereal made of 75% oat and 20% corn and rye flours; made according to the patent from a dough cooked to the boiling point of water, formed into small "donuts" which are dried and subjected to a pressure of 80 to 100 lbs. at a temperature of 190° C. to 232° C. in from $5\frac{1}{2}$ to $6\frac{1}{2}$ minutes and exploded or puffed by releasing pressure. This product is hereinafter referred to as gun exploded oats-corn-rye cereal. [3]

[2] Quaker Oaties produced under patent No. 2,233,602.

[3] Cheerioats produced under patent No. 2,162,376.

(4) Puffed oats made by preheating oat groats for 5 minutes to a temperature of 122° C. and then subjecting the groats to live steam up to 200 lbs. 198° C. in 2 minutes and finally puffing by releasing the pressure suddenly. This latter product has been sold in limited amounts here and in Canada.

EXPERIMENTAL

The method of measuring protein quality used in these experiments is a modification of the original rat growth method of Osborne, Mendel and Ferry ('19) in which the gain in body weight per gram of protein consumed is used as an index of the protein quality of the material under examination.

Animal test groups were made up of three male and three female rats from 50 to 60 gm. in weight. Each test animal was kept in an individual screen bottom cage in an air conditioned room.

The test materials were incorporated into a basal ration in amounts that would bring the total protein (N $\times$ 6.25) to 10% of the total ration. Since the basal ration had 0.5% protein, the test material supplied 9.5% protein to the ration. The other ingredients included 4% salt mixture (Hawk and Oser, '31), 2% cod liver oil, 1.5% vitamin B complex powder [4] and the balance carbohydrates. The vitamin B complex powder furnished the following vitamins per 100 gm. of basal ration: 0.9 mg. of B_1, 0.27–0.32 mg. of B_2, 0.26 mg. of B_6, 1.1–1.4 mg. of pantothenic acid. Rations 22 to 25, inclusive, were supplemented with synthetic vitamins to contain the following levels of vitamins in 100 gm. of ration: 0.9 mg. of B_1, 1.6 mg. of B_2, 1.2 mg. of B_6, 1.2 mg. of pantothenic acid, and 2.5 mg. of alpha-tocopherol.

A summary of the performance of the individual animals at the termination of the 56-day test period is given in table 1, part A. In tabulating, the data have been placed in three groups in each of which different methods of feeding were used. In the first group rolled oats,[5] toasted oat cereal

[4] Yeast concentrate No. 200 QGS Vico Products Company.
[5] Quaker Oats.

TABLE 1

Performance of individual rats on 10% protein rations at the end of a 56-day test period.

RAT	SEX	INITIAL BODY WEIGHT	GAIN IN BODY WEIGHT	RATION EATEN	AVERAGE DAILY INTAKE	GAIN PER GRAM OF PROTEIN	RELATIVE PROTEIN QUALITY
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
			A All groups fed ad libitum.				
		Group 9 Rolled oats ration (10% protein, 5.4% fat)					
1	male	59	105	585	10.4	1.80	
2	male	56	96	616	11.0	1.57	
3	male	59	118	692	12.4	1.71	
4	female	55	87	652	11.6	1.56	
5	female	58	94	610	10.9	1.55	
6	female	57	79	759	13.6	1.05	
Average		57	97	637	11.4	1.52	100
		Group 12 Oven expanded cereal ration (9.5% protein, 3.4% fat)					
1	male	59	79	500	8.9	1.67	
2	male	55	96	572	10.2	1.77	
3	male	58	95	577	10.3	1.74	
4	female	57	67	486	8.7	1.46	
5	female	57	73	558	10.0	1.39	
6	female	58	72	506	9.0	1.51	
Average		57	80	533	9.5	1.60	105
		Group 15 Drum dried oatmeal ration (10.1% protein, 4.1% fat)					
1	male	56	78	516	9.2	1.50	
2	male	55	71	463	8.3	1.52	
3	male	55	81	503	9.0	1.59	
4	female	61	83	535	9.6	1.54	
5	female	60	108	699	12.3	1.53	
6	female	52	76	462	8.3	1.63	
Average		57	82	530	9.5	1.55	102
		B Group 13 fed ad libitum Daily intakes of groups 14 and 10 restricted to that of group 13					
		Group 13 Gun exploded oats corn rye cereal ration (9 7% protein, 3.7% fat)					
1	male	56	13	324	5.8	0.42	
2	male	58	11	327	5.8	0.35	
3	male	57	20	484	8.6	0.43	
4	female	55	18	357	6.4	0.52	
5	female	58	20	438	7.8	0.47	
6	female	59	16	393	7.0	0.42	
Average		57	17	387	6.9	0.44	39
		Group 14 Rolled oats ration (9 8% protein, 5 4% fat)					
1	male	61	40	388	6.9	1.05	
2	male	63	42	388	6.9	1.10	
3	male	57	51	388	6.9	1.34	
4	female	54	41	388	6.9	1.08	
5	female	62	39	388	6.9	1.03	
6	female	60	47	388	6.9	1.24	
Average		60	43	388	6.9	1.14	100
		Group 10 Home cooked oatmeal ration (10% protein, 5 4% fat)					
1	male	57	46	388	6.9	1.19	
2	male	55	49	388	6.9	1.26	
3	male	55	48	388	6.9	1.24	
4	female	54	47	388	6.9	1.21	
5	female	57	41	388	6.9	1.06	
6	female	58	45	388	6.9	1.16	
Average		56	46	388	6.9	1.19	104

and drum dried oatmeal were tested. The animals were fed ad libitum and a record of the food intake and weight changes was kept.

Although the average protein efficiency (gain/gram protein) of these three rations is practically constant there is considerable variation between individual rats within a group which may be attributed to the differences in food intake. In order to eliminate this variable in the succeeding protein quality tests the food consumption was equalized between animals and groups by limiting the amount of food given each animal to the average daily consumption of the group eating the least amount of food.

In the second experiment a ration containing gun-exploded oats-corn-rye cereal was fed ad libitum. In preliminary experiments it was found that animals on this ration tended to consume small amounts of food whereas on rolled oats their voluntary consumption was much greater. Therefore, the animals on the rolled oats and "home" cooked oatmeal rations were given a daily allowance equivalent to the average consumption of the gun-exploded cereal group on the preceding day. A summary of the performance of these animals is given in table 1, part B.

In this experiment the animals within each group show less biological variation than the animals on the unrestricted ration. It will be noted, however, that there is quite a drop in the protein efficiency of the rolled oats in the second experiment. This drop is due in part to the greatly lowered food intake in the second experiment.

It should also be pointed out that the animals on the gun exploded oats-corn-rye cereal ration tended to eat less food as the test period proceeded. This worked to the definite disadvantage of animals on other rations that showed fairly good growth responses at the beginning of the test period, and were then limited to smaller amounts of food even though their maintenance requirements had become greater. Partial starvation resulted and the protein efficiency, when calculated

over the entire feeding period, was lower than would be expected if a more balanced feeding program had been adopted.

From previous experience it was estimated that the animals on the gun exploded oats-corn-rye cereal ration would consume the least amount of food in the final series of tests and that their average daily consumption for the 56-day period would be approximately 7.0 grams. Accordingly all the animals in each group were offered 7.0 grams of their test rations each day during the test period.

The four materials tested were: rolled oats, exploded oats-corn-rye cereal, puffed oats and a 7.5 to 1 to 1 mixture of rolled oats, corn and rye flour respectively. As nearly as can be determined this latter mixture has the same cereal ingredients as the exploded oats-corn-rye cereal but has not been heat processed. In this test, the fat levels of all the rations as determined by the acid hydrolysis method were adjusted to approximately 11% by the addition of lard. Vitamins B_2, B_6 and alpha-tocopherol were also added to eliminate the possibility of a deficiency from these dietary essentials.

The results of this experiment given in table 2 confirm those of the previous test and show that the nutritive value of oat protein is definitely impaired, presumably by the severe heat treatment encountered in the explosion method.

Although three different methods of feeding have been used in these tests, it is possible to evaluate the various methods of processing since a common factor, rolled oats, appears in all three experiments. Arbitrarily assigning the value 100 to rolled oats in each of the three experiments the relative values of the other oat products can be calculated from the gain per gram of protein consumed. The values are given in the final column of tables 1 and 2.

If these values are a true indication of the relative protein quality, it can be concluded that normal cooking does not impair protein quality but may actually slightly improve the cereal. On the other hand severe heat treatment, such as is encountered in the explosion process destroys the quality of oat protein to a large extent.

Performance of individual rats on 10% protein rations.
All animals offered 7.0 gm. of test ration each day of 56-day period.

RAT	SEX	INITIAL BODY WEIGHT	GAIN IN BODY WEIGHT	RATION EATEN	AVERAGE DAILY INTAKE	GAIN PER GRAM OF PROTEIN	RELATIVE PROTEIN QUALITY
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	
		Group 22	Rolled oats ration (10% protein)				
1	male	57	58	392	7.0	1.48	
2	male	54	65	392	7.0	1.66	
3	male	58	62	392	7.0	1.58	
4	female	54	59	392	7.0	1.50	
5	female	54	62	392	7.0	1.58	
6	female	60	51	392	7.0	1.30	
Average		57	60	392	7.0	1.52	100
		Group 23	Untreated oats-corn-rye ration (10.2% protein)				
1	male	55	53	392	7.0	1.42	
2	male	58	54	392	7.0	1.45	
3	male	54	53	392	7.0	1.42	
4	female	55	55	392	7.0	1.48	
5	female	57	58	392	7.0	1.56	
6	female	55	59	392	7.0	1.58	
Average		56	55	392	7.0	1.48	97
		Group 24	Gun exploded oats-corn-rye cereal ration (9.5% protein)				
1	male	57	18	366	6.5	0.48	
2	male	55	20	386	6.9	0.51	
3	male	58	18	362	6.5	0.49	
4	female	56	17	387	6.9	0.43	
5	female	57	13	362	6.5	0.35	
6	female	56	24	391	7.0	0.60	
Average		57	18	376	6.7	0.48	32
		Group 25	Puffed oats ration (9.8% protein)				
1	male	57	15	390	7.0	0.39	
2	male	52	11	354	6.3	0.32	
3	male	53	13	322	5.7	0.41	
4	female	52	10	349	6.2	0.29	
5	female	55	11	374	6.7	0.30	
6	female	54	11	338	6.0	0.33	
Average		54	12	354	6.3	0.34	23

SUMMARY

Several oat products processed by different commercial procedures, as well as home cooked oatmeal, have been tested for their relative protein quality by a rat growth method. The protein efficiency calculated from the gain in body weight

per gram of protein consumed indicates ordinary household preparation of oatmeal, precooking of oat flour by the drum dried process and toasting of an extruded oat flour product does not cause any impairment of the protein quality. Processing by means of the explosion technique does cause considerable damage to the protein quality of oat products.

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DETERMINATION AND CONTENT OF CAROTENE AND VITAMIN A IN WISCONSIN BUTTER[1]

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ONE FIGURE

Most methods for determining carotene and vitamin A in butter and milk involve the use of a spectrophotometer (Baumann and Steenbock, '33; Semb, Baumann, and Steenbock, '34; Olson, Hegsted, and Peterson, '39). Willstaedt and With, '38, developed a method based on the reaction of vitamin A with antimony trichloride and measured the amount of color product with a step-photometer. Koehn and Sherman ('40) also utilized the antimony trichloride reaction for A but used the photoelectric colorimeter for the measurement. Carotene was also determined by means of this instrument. A modification of the Koehn and Sherman procedure was adopted by the Technical Comittee, and this method, with some changes in details, is that employed in this paper.

STANDARD CURVES FOR CAROTENE AND VITAMIN A

Beta-carotene [2] (M. P. 180° C.) was used for the construction of a standard curve with galvanometer readings given by passing the light through a 440 mµ. filter. Ten dilutions of carotene, ranging from 0.2 to 4 µg./cc. were made and read. With galvanometer readings (G_{440}) above 46 [3] the curve is represented by the factor 2.93. In this range $L_{440} \times 2.93 = $ µg. carotene per cc. L values corresponding to galvanometer readings ($L = 2 - \log G$) are supplied with the colorimeter. Below 46^3 the curve is no longer straight and the factor increases with concentration of carotene.

The standard curve for vitamin A was made with crystalline vitamin A alcohol.[3] It was stated to have an extinction coefficient of 1720. Eight dilutions of the alcohol ranging from approximately 1 µg. to 8 µg./cc. were treated with the Carr-Price reagent, the resultant color read through a 660-mµ. filter, and the readings used in the construction of the curve. The factor relating weight of vitamin A to the corresponding L value was 1.33 ± .06. Hence $L_{620} \times 1.33 = $ µg. vitamin A/cc.

CHROMATOGRAPHIC SEPARATION OF PIGMENTS

The naturally-occurring non-carotene pigments of butter as well as butter colors can be separated from the carotene by chromatography. The results thus obtained were used as the standard in developing the solvent-separation method. Heavy U.S.P. magnesium oxide, tested for its retention of β-carotene, was dry-packed in a tube to form a column 4 to 5 cm. long and 1.25 cm. in diameter. The solution of pigments in Skelly Solve B (hereafter called S.S.B.) was concentrated under reduced pressure to about 5 cc. and forced into the column with compressed air. The column was washed with S.S.B. until the solvent came through colorless and the fraction read as carotene. The non-carotene pigments were then eluted from the column with S.S.B. containing 10% absolute alcohol. This solution was evaporated to dryness under reduced pressure, the residue was taken up in S.S.B., and the solution read for non-carotene pigments expressed as carotene.

[2] Obtained from the S.M.A. Corporation, Cleveland, Ohio.
[3] Obtained from Distillation Products Company, Rochester, N. Y.

Diacetone alcohol diluted with a small quantity of water to 94% strength was found to remove more non-carotene pigment from S.S.B. solutions than does 92% methanol or 85% ethanol. The values obtained by the diacetone extraction procedure agreed closely with the chromatographic results.

TABLE 1

Carotene determination of colored and uncolored butters by different methods.

SAMPLE	METHOD	TOTAL PIG-MENT AS CARO-TENE	CARO-TENE	NON-CARO-TENE	RECOV-ERY OF TOTAL PIG-MENT	CARO-TENE
		$\mu g/gm.$ fat	$\mu g/gm$ fat	$\mu g./gm$ fat	%	% of total
1a. No added color	Chromatograph	2.65	1.66	.94	98.2	62.7
1b. No added color	94% Diacetone extraction	...	1.64	...	...	61.8
1c. No added color	92% Methanol extraction	...	1.96	...	...	74.0
1d. No added color	85% Ethanol extraction	...	2.10	...	...	79.1
2a. No added color	Chromatograph	9.73	7.48	1.76	95	77
2b. No added color	94% Diacetone extraction	...	7.23	...	...	74.4
2c. No added color	92% Methanol extraction	...	7.72	...	...	79.5
3a. No added color	Chromatograph	9.03	7.0	1.7	96.3	77.5
3b. No added color	94% Diacetone extraction	...	6.87	...	...	76.3
3c. Added color	94% Diacetone extraction	13.35	6.83	...	...	75.7

The determinations were run on S.S.B. solutions of the butter extract obtained as described later in the paper. An aliquot was read for total pigment. A second aliquot of 20 cc. was extracted with three 10-cc. portions of diacetone, washed once with 10 cc. of water, made up to volume and read. A third aliquot was chromatographed, and a fourth was extracted with three 10-cc. portions of 92% methanol, washed three times with 10-cc. portions of water, and dried over anhydrous sodium sulfate before bringing up to volume and reading. Table 1 shows typical results obtained with both colored and uncolored butters. For all samples the diacetone figures ranged from 95 to 103% of the chromatographic values and the methanol results varied from 98 to 118%. When the carotene content was exceptionally low, as in winter butters, figures as high as 118% were obtained with methanol. These high figures are probably due to the presence of acid-formed pigments of silage. These pigments pass into the milk and appear in the butter (Peterson, Bird, and Beeson, '37; Quack-

enbush, Steenbock, and Peterson, '38). Diacetone extracts these pigments while methanol does not.

Tests were made to determine the number of washings required to remove diacetone and methanol from the S.S.B. solution of carotene. One washing proved sufficient to remove the cloudiness caused by these solvents. Three to six washings had no effect on the quantity of carotene found.

Drying the S.S.B. solution of carotene over anhydrous sodium sulfate before reading appears not only to be unnecessary but may yield erroneous results. In no case were the readings of the dried carotene solutions higher, and on several butter samples they were lower than the values for undried and chromatographed samples. These losses were later traced to the kind of Na_2SO_4 used. One, a granular product, absorbed no carotene but a second, which was very finely powdered, removed from 8 to 22% of the total carotene from solution. No such absorption of carotene or vitamin A from ether solution by anhydrous sodium sulfate was encountered.

COMPOSITION OF PIGMENTS IN THE SOLVENT FRACTIONS

The composition of the fractions obtained by extracting the S.S.B. solution of pigments with diacetone and methanol was determined by chromatographing. Before chromatographing, the extracted S.S.B. solution was washed with water to remove the interfering methanol and diacetone. The pigments in the methanol and diacetone extracts were transferred to S.S.B. for chromatographing by diluting the extracts with an equal volume of water and shaking with three 10-cc. portions of S.S.B. Table 2 contains the results of a typical analysis.

The data show that approximately 10% of the total carotene (0.64 µg. out of 6.5 µg.) was removed by the diacetone-alcohol but that non-carotene pigment (0.38 µg.) was left behind to compensate largely for this removal. Extraction with 92% methanol removed only 0.1 µg. of carotene but left behind about 0.95 µg. of non-carotene pigments. The carotene

figure by the diacetone method (6.33) checks reasonably well with the chromatographic figure (6.5), but the methanol value (7.22) is 11% too high.

TABLE 2

Chromatographic analysis of diacetone and methanol fractions.

NO.	FRACTION	TOTAL PIG- MENT AS CAROTENE	CAROTENE	NON- CAROTENE	RECOVERY	CAROTENE IN FRACTION
		$\mu g./gm.$ fat	$\mu g/gm.$ fat	$\mu g./gm.$ fat	%	% of total
1.	Skelly Solve B solution of total pigments	8.65	6.5	2.12	99.7	100
2.	Residue after extraction of 1 with diacetone	6.33	5.77	0.38	97.1	88.8
3.	Diacetone extract of 1	2.32	0.64	1.66	99.1	9.9
4.	Residue after extraction of 1 with 92% methanol	7.22	6.3	0.95	100.4	96.9
5.	Methanol extract of 1	1.43	0.1	1.17	88.8	1.5

CORRECTION FACTORS FOR PRESENCE OF CAROTENE AND ADDED BUTTER COLOR OF VITAMIN A

Antimony trichloride reacts with carotene to produce a blue color with maximum absorption at 590 mµ. but with sufficient absorption at 620 mµ. to cause an error in the calculation of A if no correction is made. A correction factor was arrived at by testing six dilutions of a carotene solution ranging from approximately 1 µg. to 11 µg./cc. The correction factors ranged from 0.0081 to 0.0059 increasing as the concentration decreased. The average of the six values was 0.007. This factor is an expression of the effect on the L value at 620 mµ. when 1 µg. of carotene reacts with antimony trichloride. It is applied by multiplying the µg. of carotene present in the Evelyn tube by 0.007 and subtracting this from the observed L value.

The dyes yellow AB and OB also react with antimony trichloride to produce a pink color with some absorption at

620 mμ. The same correction factor was found applicable to them as is applied to carotene. Therefore:

L_{620} (corrected) $= L_{620}$ (observed) $- (0.007 \times$ μg. total pigment expressed as carotene).

SOLVENT EXTRACTION METHOD FOR ANALYSIS OF BUTTER

Whole butter may be used if the fat content is known. If not, the butterfat is obtained by melting the butter on a water bath below 60° C. and filtering it through cotton. It is then allowed to solidify in the refrigerator. The cake is removed and scraped free of any adhering particles of moisture and non-fat solids. Ten gm. of fat are weighed into a suitable saponification flask. Five gm. of solid KOH, 5 cc. of water and 20 cc. of aldehyde-free methanol are added, and the flask is connected by a ground glass joint to a condenser and refluxed on a water bath for 10 minutes. The sample is then diluted with 40 cc. of distilled water, transferred to a separatory funnel, and the flask rinsed with an additional 40 cc. of water. The mixture is extracted with one 100-cc. and two 50-cc. portions of peroxide-free ether. The combined extracts are washed with 100-cc. portions of distilled water until the washings are free of alkali. Four washings should be sufficient. The ether solution is dried over anhydrous sodium sulfate and transferred to a round-bottom Claisen flask; the sodium sulfate is washed three times with 10-cc. portions of ether and the washings added to the main solution. Several glass beads are added to prevent bumping. The flask is placed on a warm water bath and the ether removed under reduced pressure. Care must be taken not to continue heating the flask after the ether has been removed. About 15 cc. of S.S.B. is added before the vacuum is released. If the flask is still warm, it is cooled under the water tap. The solution and rinsings of the flask with S.S.B. are transferred to a 50-cc. volumetric flask and made up to volume.

A 5-cc. aliquot is diluted to 10 cc. and read for total pigment. Another aliquot of 25 cc. is extracted with three 10-cc. portions of 94% diacetone-alcohol to remove non-carotene

pigments, washed once with 10 cc. of water, made up to 50 cc. with S.S.B. and read for carotene.

For the vitamin A determination 1 cc. of the original S.S.B. solution is placed in an Evelyn tube, the S.S.B. is removed under vacuum and replaced by 1 cc. of chloroform. Nine cubic centimeters of a 20% solution of antimony trichloride in chloroform are added from a pipette with a delivery time of about 2 seconds. The galvanometer is read at the point of temporary stability.

The volumes given for carotene and vitamin A are suitable for summer butters, but for winter butters, which have less pigment, a greater concentration of the final solution may be required.

The instrument used is the Evelyn Photoelectric Colorimeter with a 440-mμ. filter for carotene and a 620-mμ. filter for vitamin A. Concentrations are read off standard curves and the proper correction factors applied.

The above method differs from that adopted by the Technical Committee in certain respects, the most important being the following:

In the Committee method the extracting solvent for removal of non-carotene pigments is 92% methanol except for winter butters produced by cows fed acid or molasses silages. For such butters 94% diacetone is recommended. Since in most cases the kind of ration is not known, 94% diacetone has been used for all samples.

In the Committee method the S.S.B. solution used for the carotene determination is washed four times with water and dried over sodium sulfate before being diluted to a suitable volume. In our method one washing and no drying is used. Our data on the effect of washing and drying did not reach the Committee in time for consideration before the formulation of the method.

CAROTENE AND VITAMIN A CONTENT OF WISCONSIN BUTTERS

Early in April, 1942, twenty-two 1-lb. samples of butter made during the week of March 23, 1942, by creameries in

southwestern Wisconsin were analyzed. In July, 1942, twenty
samples of butter (fifteen from the same creameries, five
from others in the same region) were analyzed. Table 3 sum-
marizes these analyses.

TABLE 3

Carotene and vitamin A content of Wisconsin butters

	SERIES I		SERIES II	
	March, 1942	July, 1942	Sept., 1942	Jan., 1943
Number of samples	22	20	70	65
Range, µg./gm. butterfat				
Total pigment	. . . .	7.2 — 13.6	6.2 — 14.8	. . . .
Carotene	1.9 — 2.7	5.4 — 9.6	4.8 — 11.0	2.0 — 4.9
Vitamin A	4.6 — 6.8	7.4 — 10.5	5.9 — 11.3	4.2 — 7.2
Mean, µg./gm. butterfat				
Total pigment	. . . .	10.33	10.53	. . . .
Carotene	2.16 ± .04 [1]	7.43 ± .26	7.66 ± .16	2.97 ± .06
Vitamin A	5.61 ± .14	8.96 ± .19	9.06 ± .14	5.89 ± .07
International units per lb. of butter	9,568	17,738	18,032	10,488

[1] Standard deviation of the mean.

With the aid of the Division of Marketing of the State
Department of Agriculture, collection and analysis of Wis-
consin butters was undertaken on a much larger scale in
September, 1942, and January, 1943. The state was divided
into nine districts, and from each the number of samples
corresponding to the district's proportion of the state pro-
duction were collected and analyzed. Inspectors of the Wis-
consin Department of Agriculture collected these samples
from the creameries, packed them in ice or dry ice in most
cases,[4] and sent them directly to the laboratory together with
data sheets regarding the samples. The data sheets asked
for as much information (e.g., data on churning, breed of
cattle, rations, etc.) as was thought pertinent in interpreting
the results.

Samples from seventy different creameries were collected
in September, 1942, and other samples from sixty-five of these

[4] The packing was probably not necessary. Several samples kept at room tem-
perature for 3 days, which is longer than a sample would be in transit, showed no
change in carotene or vitamin A content.

creameries were collected in January, 1943. The analyses of these samples are also summarized in table 3. The total pigment values are not given for the winter butters since these are usually artificially colored and therefore the figures would be of little significance.

In the calculation of International Units 0.6 µg. of β-carotene and 0.25 µg. of vitamin A were each taken as equal to 1 I.U. (Baxter and Robeson, '42). The average butterfat content of the butter was assumed to be 81%.

The table shows the carotene content of July butters as almost 3.5 times that of March butters, and the vitamin A content about 1.6 times as great. September values do not vary greatly from those of July. The January butters, however, show a slightly higher content of vitamin A and a considerably higher carotene content than the March samples. From September to January the carotene content decreased about 60%, while the vitamin A decreased about 35%. About 75% of the total pigment of the summer butters was carotene. The few samples of uncolored winter butters which were obtained contained 60 to 65% of the total pigment as carotene.

The distribution of the September and January samples according to their carotene and vitamin A content is shown in figure 1. The September samples show an approximately normal type of distribution, but the January samples show a much narrower spread. This concentration effect is probably related to the storage of carotene and vitamin A in the tissues of cattle and to the low content of carotene in winter rations. During the winter feeding, cattle draw on these stores to hold up the carotene and vitamin A content of the milk. Only after several months does the milk reach a constant low level. The drop in vitamin A is less than that for carotene, because the latter is constantly being converted into the former and hence acts as a reservoir for it.

The distribution of the March samples (not shown in the figure) was of the same type as in January. However, there was a further general decline with the majority of the samples

falling in the next lower divisions as compared with the January distribution.

Variations among samples at any time may be related to several factors, but breed and carotene content of the ration are probably the two most important. Samples high in carotene and low in vitamin A invariably came from districts where 70% or more of the cattle were reported to be Guernseys. In districts where Holsteins predominated, the opposite relationship existed. About ten samples for each group were obtained from districts with this preponderance of one or

NUMBER OF SAMPLES

SEPTEMBER   JANUARY

☑ CAROTENE
□ VITAMIN A

μG. OF CAROTENE OR VITAMIN A/GM. BUTTERFAT
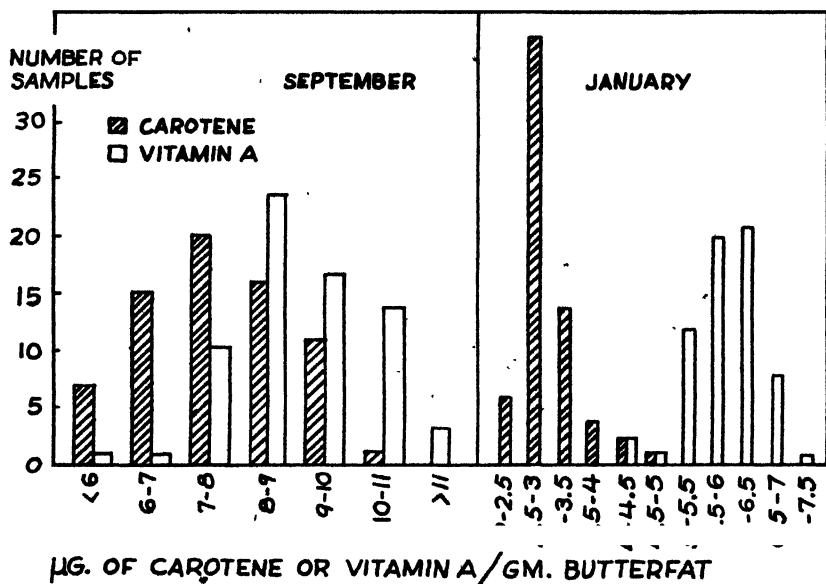
Fig. 1 Distribution of September and January butter samples according to their carotene and vitamin A content.

the other breeds. In the September series micrograms of carotene and vitamin A per gram of butterfat averaged as follows: Guernsey 9.47 and 7.34; Holstein 5.66 and 10.39. Corresponding figures for the January series were: 3.85, 5.48; 2.51, 6.50. These results bear out previous reports by different investigators on the effect of breed.

The butters were scored by Dr. L. C. Thomsen of the Department of Dairy Industry, but no correlation was found between the vitamin content and the score assigned.

In 1938 and 1939 this laboratory made a survey of the carotene and vitamin A content of market milks of the state (Dornbush, Peterson, Olson, '40). Calculating on the basis of butterfat content and correcting for the inclusion of non-carotene pigments in the milk survey values, it appears that the milks of that period were about 18% higher in vitamin A activity than the present butters. However, since the milks showed a variation of about 15% for corresponding seasons in the 2 years, this difference is not significant.

EFFECT OF STORAGE OF BUTTERS ON VITAMIN A CONTENT

In June, 1942, four samples of butter produced by the Department of Dairy Industry were analyzed prior to being placed in storage at − 21 to —23° C. These butters were stored in conventional 64-lb. unparaffined tubs lined with parchment paper and closed with wooden lids. In February they were taken out of storage and again analyzed. No loss of carotene or vitamin A was observed. The seventy 1-lb. samples obtained in September, left in the original paper wrapping and carton, were also placed in storage. Ten of these were analyzed in February, 1943. Again no loss of either carotene or vitamin A was observed.

SUMMARY

A solvent extraction method is described for the determination of carotene and vitamin A in butters, utilizing the yellow color of the carotene for its determination and the Carr-Price reaction for the determination of vitamin A.

Diacetone alcohol, 94%, proved to be superior to 92% methanol for the extraction of non-carotene pigments.

The March butters averaged about 9,500 I.U./lb. The butters of July and September averaged about 18,000 I.U./lb. The January butters were slightly better than the March butters and contained about 10,500 I.U./lb.

In the summer butters, about 75% of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65% of the pigment was carotene.

Storage of butter as long as 8 months did not result in a loss of carotene or of vitamin A.

ACKNOWLEDGMENT

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THE ASCORBIC ACID REQUIREMENTS OF SCHOOL-AGE GIRLS [1]

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ONE FIGURE

During a recent investigation in this laboratory (Roberts and Roberts, '42) concerning the vitamin C requirements of children, five subjects were tested on intakes ranging from 55 to 135 mg. On the basis of blood and urinary excretion studies, it was found that 65 to 75 mg. of ascorbic acid was needed to insure saturation of these children. It seemed pertinent to extend the investigation to include a larger number of subjects in order to determine whether the majority of children in a larger group would respond in the same way.

It is well known that many children rarely get as much as 65 mg. of ascorbic acid in the diet. The subjects in this study were tested, therefore, on lower levels of intake than had been used in the earlier study.

EXPERIMENTAL

Selection of subjects. This investigation was carried on at a suburban home for girls. Thirty pre-adolescent girls between the ages of 6 and 12 years, living in two cottages, were selected as subjects. These children were in a healthy

condition although, as in most groups of children, some were underweight, and some overweight. This deviation from average weight for height and age was taken into consideration, however, in the grouping of the children.

General procedure. The subjects were divided into six groups as based upon original ascorbic acid levels of the blood, age, height, weight, and deviation from average weight. The children from each group were given daily supplements of crystalline ascorbic acid [2] for three 7-day periods. The supplements ranged from 10 mg. for the first group to 60 mg. for the sixth group.

The amount of reduced ascorbic acid in the blood plasma was determined each week. At the end of the third period, the urinary "resting level" of ascorbic acid and the response to a 300-mg. test dose were measured. The blood concentration and the response to the test dose were the criteria used in judging the state of saturation of the subjects.

Control of the diet. Determination of the dietary intake for six of the subjects over a weekly period showed that the children were getting between 31 and 94 mg. of ascorbic acid with an average of 59 mg. On this intake their blood levels ranged from 0.46 to 0.97 mg. per 100 cc.

Because of the generous amount of vitamin C-rich foods served in the institution on some days, it was necessary to make a few dietary adjustments. Tomatoes, fresh and canned citrus fruits, all raw vegetables except carrots, and all fresh fruits except apples were eliminated from the diet. The menus were adjusted so that milk and dried fruit were the only sources of ascorbic acid for breakfast. Substitutions were made in the supper so that it, too, contained very little vitamin C. Most of the ascorbic acid was obtained at the noon meal where two or three vegetables and sometimes canned fruit were served. The children did not have access to food between meals.

In order to determine the daily intake of ascorbic acid, the amount of food eaten by six of the subjects at the noon meal was weighed and samples of the food were analyzed. The milk and dried fruit from breakfast were also analyzed for vitamin C while the intake from the evening meal was estimated partly by analysis, and partly from tables of average composition. Inasmuch as the plates were served from the kitchen where the food had been divided quite evenly, and the children were required to "clean their plates," it seemed that the average obtained in this way would be fairly representative of the dietary intake of all subjects.

During the last 2 days of the study when urinary excretions were being determined, the diet was strictly controlled, and all foods were weighed and analyzed. In this way the intake of foods containing any vitamin C was kept the same for each subject. The average intake during each of the three weekly periods was 22, 21, and 22 mg.

Urinary determinations. The daily urinary output was collected for the last 2 days of the investigation, one day when the children were receiving their regular dietary supplement, and the other when they were given a 300-mg. test dose. To preserve the ascorbic acid in the urine, 5 N sulphuric acid and 8-hydroxyquinoline were used as suggested by Sendroy and Miller ('39).

Chemical methods. For the food and urine analyses, the method of Bessey and King ('33) was used. A buffer was added to the acidified urine to prevent the rapid fading of the end point. For blood determinations the microtechnique with the Farmer and Abt apparatus ('36) was used. All determinations were made on fasting blood plasma. The procedure of Menaker and Guerrant ('38) was employed for the standardization of the indicator, 2,6-dichlorophenolindophenol.

RESULTS AND DISCUSSION

In the present study, two criteria have been used for interpreting the results in terms of ascorbic acid requirements.

These are the same as were used in the more detailed study of Roberts and Roberts ('42). Blood levels of 0.7 mg. per 100 cc., and an excretion of 50% of a 300-mg. test dose within 24 hours are the two indices used to indicate a satisfactory state of nutrition in respect to vitamin C. Inasmuch as the daily excretion was obtained for only 1 day ascorbic acid "retention", which was one criterion in the earlier study, has not been considered in this report.
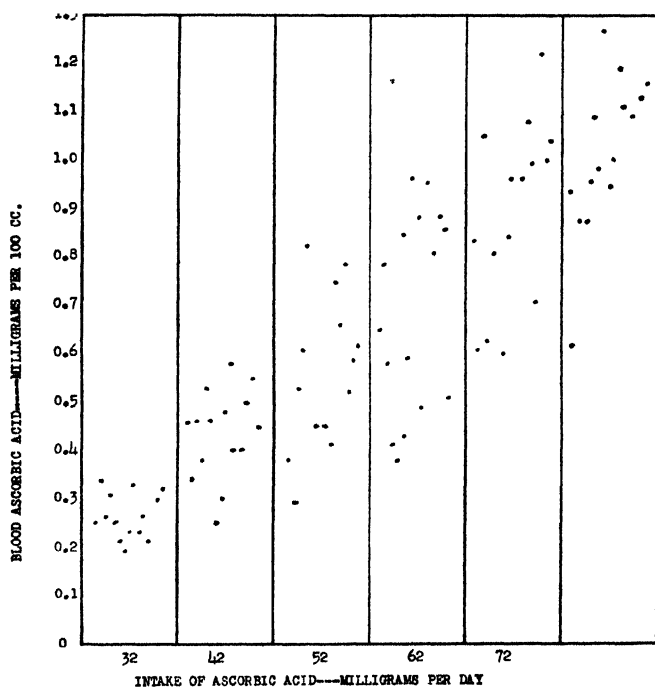
Fig. 1   The concentration of ascorbic acid in the blood plasma on various levels of intake. The figure shows results of three tests made at weekly intervals on each of thirty children.

A wide variation in blood ascorbic acid concentration on each level of intake is shown in figure 1. It will be noted that the blood levels are less variable on the lower than on the higher intakes. This variability has been observed by others. With boys living on an institution diet Roberts et al. ('39)

report blood levels ranging from 0.4 to 1.4 mg. per 100 cc. Bessey and White ('42) likewise found "considerable scattering" of blood concentrations at any one level of intake. No doubt these wide ranges are in part due to individual eating habits, and in part to variability in the response to vitamin C. Even with a controlled intake the individual blood

TABLE 1

Blood levels and excretion of ascorbic acid on various levels of intake.

GROUP	INTAKE		SUBJECT	BLOOD LEVELS MG. PER 100 CC.				EXCRETION	
	Average from food	Supplement		Periods			Average for 3 periods	"Resting level"	After 300 mg. test dose
				1	2	3			
	mg.	*mg.*						*mg*	*%*
I	22	10	1	0.34	0.25	0.23	0.27	9	7
			2	0.25	0.21	0.26	0.24	7	15
			3	0.34	0.23	0.21	0.26	9	4
			4	0.26	0.19	0.30	0.25	8	22
			5	0.31	0.33	0.32	0.32	7	6
II	22	20	6	0.34	0.46	0.40	0.40	8	14
			7	0.46	0.25	0.40	0.37	10	22
			8	0.46	0.30	0.50	0.42	12	32
			9	0.38	0.48	0.55	0.47	14	39
			10	0.53	0.58	0.45	0.52	8	37
III	22	30	11	0.88	0.83	0.79	0.83	14	72
			12	0.38	0.45	0.52	0.45	10	77
			13	0.29	0.45	0.41	0.38	27	8
			14	0.53	0.75	0.59	0.62	13	90
			15	0.61	0.66	0.62	0.63	14	46
IV	22	40	16	0.65	0.85	0.96	0.82	23	78
			17	0.79	0.59	0.81	0.73	21	68
			18	0.58	0.97	0.89	0.81	28	65
			19	0.41	0.89	0.86	0.72	22	58
			20	0.38	0.49	0.51	0.46	10	32
V	22	50	21	0.84	0.60	1.05	0.83	31	71
			22	0.61	0.85	0.71	0.72	42	72
			23	1.06	0.97	1.23	1.09	34	79
			24	0.63	0.97	1.01	0.87	33	71
			25	0.81	1.09	1.05	0.98	14	75
VI	22	60	26	0.94	1.10	1.20	1.08	27	81
			27	0.62	0.94	1.12	0.89	..	..
			28	0.88	1.28	1.10	1.09	44	83
			29	0.88	0.95	1.14	0.99	22	45
			30	0.96	1.01	1.17	1.05	26	83

levels vary. With five subjects on the same diet Roberts and Roberts ('42) found differences as great as 0.3 mg. per 100 cc.

The blood levels and the urinary response of each child to a 300-mg. test dose are shown in table 1. Holmes, Cullen and Nelson ('41) have pointed out that repeated determinations of the blood ascorbic acid over a period of time constitute a more accurate measure of storage than a single determination. The blood levels during the three periods on the supplemented diet and also the mean value are included in the table. If a blood level of 0.7 mg. or above per 100 cc. is considered an indication of normal vitamin C nutrition, it is evident that the the children in groups I and II are subnormal. All of the children in these two groups were below 0.7 mg. per 100 cc. not only at the end of the first week, but during the entire 3 weeks of supplementation. Furthermore, the urinary "resting level" was low, and the response to the test dose was poor.

In the third group, only one child maintained a blood level above 0.7 mg. per 100 cc. Three in the group excreted 50% of the test dose. If the two criteria are applied as indications of vitamin C saturation, it is evident that subject 11 is the only child saturated on an intake of 52 mg.

The subjects in group IV who received 62 mg. showed a definite improvement. Though their blood levels were low during the first period, with the exception of subject 17, all blood levels increased during the second week, and these higher levels were maintained during the third period. Subject 20, however, is the only one whose mean blood level was below 0.7 mg. per 100 cc. Moreover, all except this girl excreted more than 50% of the test dose. No explanation can be offered as to the reason for this subject maintaining the low blood and excretion values. The urinary "resting level" for the other four subjects increased above the 20-mg. level, but fell short of the 40-mg. "resting level" considered by Van Eekelen and associates ('37) to be indicative of tissue saturation.

That the next two groups, V and VI, were in a good state of vitamin C nutrition is evidenced by the high blood levels, the increased excretion, and the favorable response to the test dose. All had higher blood values at the end of the third period than they had at the end of the first, and their mean blood levels were above 0.7 mg. per 100 cc. With the exception of subject 29, all in these two groups excreted more than 50% of the test dose.

Examination of the mouths of the subjects by a dentist showed that there was no relation between either the intake or the blood levels of ascorbic acid and the condition of the gums. Three weeks on a low ascorbic acid intake is evidently too short a period to expect any visible tissue changes.

The data show that under the conditions set up, 52 mg. is the lowest intake of ascorbic acid on which any of the children were saturated; only one child, however, reached saturation on this intake. Four-fifths of the subjects receiving 62 mg., and all getting 72 mg., were saturated. Thus it appears that 62 to 72 mg. would be a satisfactory level at which to set the standards for the school-age child.

The results of this study are in fair agreement with other investigations on the school-age child. As judged by excretion studies, Harris ('40) found approximately 50 mg. of ascorbic acid sufficient to saturate boys 11 to 13 years old. Pemberton ('40) reports 63 mg. for boys from 10 to 14 years old. When judged by the response to the test dose alone, it will be noted in the present study that three of the five children receiving 52 mg. of ascorbic acid were saturated. Only one of these subjects, however, had a blood level above 0.7 mg. per 100 cc. Using the two criteria, 62 mg. was found more nearly adequate.

On the basis of blood studies Bessey and White ('42) report 40 to 50 mg. daily, sufficient to maintain a high plasma concentration. The intake was estimated, however, on the consumption of citrus fruit and tomatoes only. This amount could be better compared with the supplements used in the present investigation. On this basis there is close agreement between the results of the two studies.

Furthermore, this investigation substantiates the results found in the more detailed study of Roberts and Roberts ('42), where it was found that 65 to 75 mg. were required to saturate the subjects when the same criteria as were used in the investigation were employed to judge saturation.

Moreover, the values obtained here further corroborate the allowance set up by the Committee on Food and Nutrition of the National Research Council ('41).

SUMMARY

The ascorbic acid requirements of thirty pre-adolescent girls between the ages of 6 and 12 years were studied. Blood concentrations and the urinary response to a test dose were determined on intakes ranging from 32 to 82 mg. of ascorbic acid. Blood levels of 0.7 mg. per 100 cc. and the excretion of 50% of a 300-mg. test dose in 24 hours were used as the criteria indicating a satisfactory state of nutrition in respect to vitamin C.

None of the children on intakes of 32 or 42 mg. of ascorbic acid, and only one on an intake of 52 mg. were receiving sufficient vitamin C to maintain these standards. Four-fifths of those receiving 62 mg. and all who received 72 mg. were saturated.

On the basis of this study 62 to 72 mg. of ascorbic acid would seem to be an adequate allowance for pre-adolescent girls of 6 to 12 years.

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THE EFFECT OF DIET ON THE VITAMIN A CONTENT OF THE BOVINE FETAL LIVER [1]

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In a recent general review article on vitamin A (Butt, '42), the statement is made that the vitamin A content of the liver is very low at birth, irrespective of the diet of the mother. Hart and Guilbert ('33) made a similar statement for cattle — namely, that "the livers of new-born animals tend to be comparatively low in vitamin A, irrespective of the diet of the dam during gestation". In the same paper they report that livers from four aborted calves whose dams were on a vitamin A-deficient diet contained no vitamin at all. The vitamin A content of livers of new-born and premature children has been investigated by Wolff ('32), Ellison and Moore ('37), Toverud and Ender ('35), Gaethgens ('37), Neuweiler ('36), and With ('40). All, except Gaethgens, found low reserves of vitamin A with considerable individual variation. Their material did not permit correlation of their findings with the diet of the mother. Low reserves were also observed by Debré and Busson ('34) in new-born children and puppies, by Dann ('34) in rats and rabbits, and by V. Eckelen and Wolff ('36) in kittens and new-born puppies. Dann observed no effect on the vitamin A content of the livers of new-born rats and rabbits after the mother had been on a carotene-enriched diet, but found slightly higher values after the mother had been on a vitamin A-enriched diet. These latter values showed no relation to the amount of vitamin A given to the mother.

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Recent results obtained in this laboratory with cattle on different dietary regimes indicate that the vitamin A content of fetal livers, although low, is in direct relationship to the mother's diet.

As part of a project to investigate the blood chemistry of vaccinated and nonvaccinated cows on different diets exposed to Brucella, the liver vitamin A of aborted fetuses was determined. The method described by Guilbert and Hart ('34) for estimation of vitamin A in liver tissue was modified for use with the Cenco photometer, using Wratten filter no. 47 for the reading of carotenoids and Wratten filter no. 58 for the reading of the blue color developed after addition of $SbCl_3$ in chloroform to the prepared sample. The calculation for vitamin A and carotenoid correction described by Kimble ('39) was used. The weight of the fresh liver samples was 5 gm. and although the vitamin A content of all fetal livers was very low, an extraction of the saponified material in 20 ml. of special petroleum ether (B.P., 33–36°C.) gave $SbCl_3$ reactions of distinct color, permitting the photometric measurement of differences in this material with low vitamin A content.

The mothers of the fetuses under investigation were maintained in four different dietary groups. The pasture group (P) consisted of animals which remained on non-irrigated natural grass pasture in Strawberry Canyon, Berkeley, California, throughout the year, without supplementary feed. The average blood vitamin A level of these animals was very low from September to January, when no green feed was found on the pasture.

Another group (PA) consisted of animals which were maintained on the same pasture as the P group but were fed 400,000 I.U. of vitamin A, in the form of shark-liver oil, twice a week from September through November. The average blood vitamin A level of these animals was higher than that of the P group.

One group of animals was transferred from pasture into the barn (B group) on September 1st and was maintained

there on a carotene and A-deficient diet consisting of beetpulp, cottonseed meal and rolled barley. A number of the barn animals received a shark-liver oil supplement (400,000 I.U.) twice a week from September through November (BA group). From October on, the average blood vitamin A level of the B group was lower than that of the P group, and the level of the BA group was considerably higher than that of the B group and approximately the same as that of the PA group.

The pregnant animals were exposed to Brucella abortus on October 2nd. Most of the nonvaccinated and a few vaccinated animals aborted during November and December. The values which were found for vitamin A content of the livers of the aborted fetuses are assembled in table 1.

The analysis of variance between the four groups revealed a highly significant difference between their means, with an F value of 11.8, which, for the appropriate degrees of freedom, corresponds to a P level of considerably less than 1%. The significance of differences between any pair of means due to unequal variance in the group may best be judged by the square root of the sum of squared standard errors. This method of analysis indicates that out of six possible comparisons only that involving the PA and BA groups does not reach statistical significance.

These significant differences for the four dietary groups indicate an effect of the dam's diet upon the vitamin A storage of the fetus. Since the vitamin A values of the blood of the fetuses are extremely low (0–10 I.U./100 ml., which is too low to permit reliable measurements of differences among groups), it may be assumed that only a very small fraction of the dam's vitamin A is able to pass through the placenta. However, these small amounts as reflected in the liver levels are in direct relationship to the dam's vitamin A storage. This is further substantiated by a few tests in which the vitamin A content of the dam's liver could be determined. The junior author performed a number of operations in which he removed the caudate lobe of the dam's liver under para-

TABLE 1

The vitamin A content of the fetal livers arranged in groups according to the diet of the dam. The vitamin A content of the liver of the dam in the four dietary groups.

COW NO	LIVER FROM DAM	GESTATION PERIOD	LIVER FROM FETUS
	I. U./gm	*days*	*I. U./gm.*
	B Group (Barn fed, low A diet)		
319	120.8	261	2.7
276	....	210	0.0
2	....	238	3.0
313	....	226	2.5
343	94.0	226	0.0
312	....	212	3.9
200	96.8	277 (calf still-born)	0.0
		Mean	1.7 ± 0.58
		Median	2.5
	BA Group (Barn fed, plus shark-liver oil)		
324	....	251	24.2
282	335.8	221	10.1
247	....	226	10.3
293	. ..	245	15.9
318	....	258	9.8
		Mean	14.1 ± 2.48
		Median	10.3
	P Group (Pasture fed)		
315	....	230	7.4
263	244.7	232	6.2
305	....	242	6.1
3	195.8	...	
		Mean	6 6 ± 0.34
		Median	6.2
	PA (Pasture fed, plus shark-liver oil)		
273	449.4 [1]	233	9.5
274	....	210	6.8
326	342.9 [1]	220	8.5
296	....	261	15.1
4	....	180	13.7
		Mean	10.7 ± 1.41
		Median	9.5

[1] Data from animals slaughtered in January, 4 weeks after abortion.

vertebral anesthesia. Partial hepatectomy was done within 24 hours after abortion, thus yielding samples which could be compared with the liver sample of the fetus (table 1).

Using the median values of the data presented in table 1, relationships between the vitamin A values for each pair of groups were obtained for the dams and fetuses (table 2). These relationships suggest the possibility of obtaining more data on this subject from slaughterhouse material. It would be unnecessary to know the past nutritional history of the animal — the simultaneous determination of the vitamin A content of the mother's liver and the fetus' liver would suffice to establish relationships as demonstrated above. Fetuses from

TABLE 2

Relationship between the vitamin A values of dams and fetuses.

GROUP			RATIO OF VITAMIN A AMONG	
			MOTHERS	FETUSES
Barn fed	:	Barn fed plus shark-liver oil	1 : 3.5	1 : 4.1
Barn fed	:	Pasture fed	1 : 2.3	1 : 2.5
Barn fed	:	Pasture fed plus shark-liver oil	1 : 4.1	1 : 3.8
Pasture fed	:	Barn fed plus shark-liver oil	1 : 1.5	1 : 1.7
Pasture fed plus shark-liver oil	:	Barn fed plus shark-liver oil	1 : 0.9	1 : 1.1
Pasture fed	:	Pasture fed plus shark-liver oil	1 : 1.8	1 : 1.5

mothers with similar liver vitamin A levels should show a similar vitamin A content in the liver, and the vitamin A stores of mothers and fetuses should be proportionately large or small.

Wolff ('32), Toverud and Ender ('35), and With ('40) have demonstrated that vitamin A values of children born at term do not differ from those of premature children. If such is the case in cattle also, it should be stated that the differences observed here in fetal livers from mothers with different vitamin A stores are much too small to have an effect on the welfare of the new-born normal calf; however, the differences are large enough to reflect the vitamin A status of the mother.

SUMMARY

During an experiment in which cattle were exposed to Brucella abortus, pregnant cows were separated into four dietary groups with different vitamin A intake. The vitamin A content of the livers of twenty fetuses aborted by these cows was measured. At the same time, the vitamin A storage of a few mothers in each dietary group was determined from liver samples obtained by partial hepatectomy immediately after abortion. It was found that the vitamin A content of the fetal liver, although low, was in direct relationship to the mother's diet.

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THE PROPHYLACTIC REQUIREMENT OF THE RAT FOR ALPHA TOCOPHEROL [1]

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Quantitative studies on the actual amount of tocopherol prophylactically required for all normal phenomena should be carried out for a significant portion of the life span of an animal or at any rate throughout early adult life. The requirement may be judged to be lower than is actually the case if an inevitable storage of tocopherol occurs in the tissues of normal young during intrauterine life and the first 3 weeks of postnatal existence. This study undertakes to determine the minimal daily requirement of the rat for tocopherol throughout early adult life.

Mason ('40) has carefully studied the alpha tocopherol requirement of male and female rats during the early months of life. Mothers and young were placed on a vitamin E low diet during the last week of lactation. The young were thus restricted to the vitamin E conveyed by placental and mammary transfer. The weaned young were continued on the vitamin E low diet for an additional 10 days before supplements of alpha tocopherol were given. The males were sacrificed after experimental periods of 50 to 90 days and the

testes and epididymis were studied histologically. A comparison was made with testes and epididymis which had been removed at the time of expected degeneration (after 40 to 70 days on the vitamin E deficiency diet). Females were mated with normal males after attaining a weight of 150 gm. (average experimental period 45 days) and the administration of alpha tocopherol was continued through the tenth day of pregnancy. In most instances a laparotomy was performed on the sixteenth day and the number of living fetuses observed. In some cases the females were allowed to continue until term. Under these conditions the minimal daily requirement for alpha tocopherol appeared to lie between 0.0375 and 0.0750 mg. He concluded that the needs of the two sexes were essentially the same. Lactation in the female and reproductive performance in the male were not studied.

Evans, Emerson and Emerson ('39), showed that a daily dose of 0.75 mg. of alpha tocopherol protected against sterility in the male to the age of 9 months; the present study extends this period to 16 months.

The extensive experiment herein reported was designed to establish the daily need for alpha tocopherol in the case of both sexes from weaning until somewhat before the approach of senescence. The study included growth, condition of the striated musculature, reproductive performance, and the prophylactic requirement of suckling young for tocopherol as measured by the presence or absence of muscular dystrophy.

<center>EXPERIMENTAL</center>

Twenty-one-day-old male and female rats were placed either on our standard vitamin E low ration (Diet 427 [2]) or on Diet I + lettuce at 21 days. These young were weaned from

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[2] Diet 427

Commercial casein 27	Brewer's yeast 10
Cooked corn starch 35	McCollum Salts No. 185 4
Lard ....·.....22	Cod liver oil 2

Diet without cod liver oil is let stand for 2 weeks to accelerate rancidity changes in the lard; the cod liver oil is added just before using.

stock colony mothers maintained on a natural food ration (Diet I + lettuce [3]).

Littermate brothers and littermate sisters were segregated into five groups, one of which was continued on the natural food ration (Diet I and lettuce); four groups on Diet 427 received six times weekly 40 mg. ethyl laurate or 0.10 mg., 0.25 mg., and 0.75 mg. a-tocopheryl acetate dissolved in 40 mg. ethyl laurate respectively.

Ten males and thirty females were started in each group. Fifteen females were used for the reproduction studies and fifteen were employed for growth.

The male rats were offered oestrus females at fortnightly to monthly intervals from the age of 4 to 16 months.

The requirement of the male rat for alpha tocopherol

The vitamin E low males showed a plateau in the weight curves at the age of approximately 6 months (graph 1). The rats receiving all three levels of alpha tocopheryl acetate were equal to or superior in growth to those receiving the stock rations. A careful examination of the gastrocnemius muscle at autopsy on the seventeenth month showed no instances of microscopic lesions in the musculature even in the group receiving only 0.1 mg. tocopherol daily. No evidences of motor impairment were ever seen in these animals. On the other hand, the E low animals were both "clinically" and microscopically impaired.

All vitamin E low rats were sterile after the second mating (4½ months, tables 1 and 2). This initial fertility can be attributed to storage as can be attested by the subsequent sterility noted for the group.

Five of the ten animals on the lowest level of tocopherol (0.10 mg.) were sterile after the second mating; however, three of the rats in this group were fertile until the age of

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[3] Diet I + lettuce ad libitum

Ground whole wheat	67.5	Melted butter	5
Casein	15	Calcium carbonate	1.5
Whole milk powder	10	Sodium chloride	1
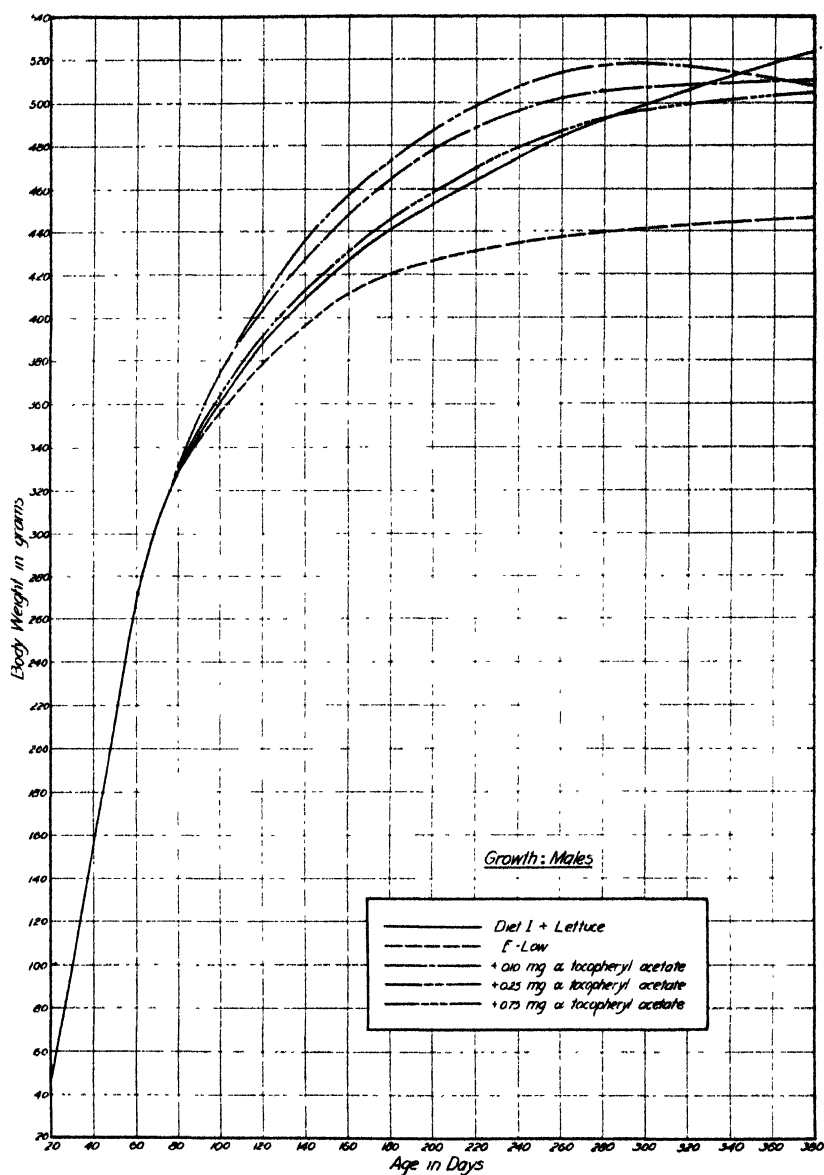
Graph 1

TABLE 1

Sexual physiology of males in the five groups.

GROUP	NO. OF RATS	ATTEMPTED MATINGS WITH OESTRUS FEMALES	INSTANCES OF PLUG OR SPERM	"REFUSED" MATINGS	FEMALES MATING THAT FAILED IMPLANTATION	NO. OF LITTERS SIRED
				%	%	
(Fourth through Ninth Month)						
E-low	10	100	87	13	93	6
0.1 mg. a-tocopheryl acetate	10	100	83	17	52	40
0.25 mg. a-tocopheryl acetate	10	100	92	8	6	87
0.75 mg. a-tocopheryl acetate	10	100	91	9	2	89
(Natural food Diet I) + lettuce	10	100	84	16	5	80
(Tenth through Fifteenth Month)						
E-low	10	100	63	37	100	0
0.1 mg. a-tocopheryl acetate	10	100	63	37	87	7
0.25 mg. a-tocopheryl acetate	10	100	88	12	77	23
0.75 mg. a-tocopheryl acetate	10	98	92	6	11	78
Natural food (Diet I) + lettuce	9	99	82	9	12	69

TABLE 2

Age of male rats in the several groups of ten each at the first sterile mating (to 16 months).

GROUP	NUMBER STERILE	AGE IN MONTHS
E-Low	5	4
	9	$4\frac{1}{2}$
	10	5
0.1 mg. alpha tocopheryl acetate	1	$4\frac{1}{2}$
	5	5
	6	$5\frac{1}{2}$
	7	8
	8	11
	10	12
0.25 mg. alpha tocopheryl acetate	4	$9\frac{1}{2}$
	7	11
	8	16
0.75 mg. alpha tocopheryl acetate	None	16
Diet I + Lettuce	None	16

11 to 12 months. Uniformly sterile matings resulted in the final 4 months (tables 1 and 2). As contrasted with the vitamin E deficient state, the lowest intake of alpha tocopherol thus had some detectable effect. On autopsy at the seventeenth month the testicles presented the appearance of little bags of water, a characteristic of the vitamin E low organ. The weights of these organs were appreciably greater than for the E low group (table 3). The histological picture of "complete degeneration" of the testicular epithelium was uniformly encountered.[4]

TABLE 3

Summary of organ weights (males — age 17 months).

	E-LOW DIET	α TOCOPHERYL ACETATE			DIET I AND LETTUCE
		0 10 mg.	0 25 mg.	0.75 mg.	
No. of rats	4	5	5	5	5
Body weight (gm.)	409	522	559	577	545
	344–476	466–594	486–670	488–736	405–664
Testes (gm.)	1.08	1.47	2.00	3.70	3.58
	0.97–1.19	1.20–1.77	1.32–2.96	3.22–4.54	3.15–4.22
Seminal vesicles (gm.)	1.88	1.90	1.79	2.01	1.43
	1.51–2.21	1.00–2.54	1.13–2.22	1.34–2.90	0.81–1.77
Prostate (gm.)	0.97	1.01	1.08	1.23	1.16
	0.70–1.21	0.78–1.19	0.86–1.45	0.85–1.70	0.76–1.35
Cowper's glands (mg.)	108	116	114	118	135
	90–142	87–134	85–129	114–123	101–217
Adrenals (mg.)	54	54	53	52	45
	48–61	48–65	47–60	44–61	25–66
Thyroids (mg.)	40	47	47	38	77
	29–53	39–59	32–69	30–47	52–99
Liver (gm.)	18.60	18.04	17.81	17.76	15.88
	15.16–22.16	16.15–21.41	15.10–21.26	14.48–23.46	11.20–20.36
Kidneys (gm.)	3.30	3.36	3.44	3.55	2.97
	2.86–4.13	3.20–3.58	2.91–4.14	3.30–3.85	2.42–3.55
Heart (gm.)	1.59	1.56	1.56	1.49	1.49
	1.35–1.84	1.27–1.85	1.33–1.86	1.36–1.63	1.16–1.78

[4] It will be noted that the accessory organs of reproduction did not show the hypertrophy reported for younger animals (Evans, Emerson and Emerson, '39).

The performance of the rats receiving 0.25 mg. of alpha tocopheryl acetate was, in general, satisfactory during the first 9 months of life. Sterility had supervened in a majority of cases by the end of the first year as is seen from tables 1 and 2.[5] The testes of the animals in this group were subnormal in weight and usually showed marked degeneration.[6]

The rats on the high level of the tocopherol (0.75 mg.) were slightly superior in reproductive behavior to their stock ration brothers during the period studied.

The results of these tests indicate that the daily requirement for alpha tocopheryl acetate, as a supplement to the vitamin E low diet (Diet 427) is more than 0.25 mg. per day and the actual figure lies between this value and 0.75 mg.

The importance of long term experiments is seen from the fact that our results indicate that the inadequacy of 0.25 mg. daily of tocopherol may in some instances not disclose itself until 9½ months. The ages of the rats in the several groups at the onset of sterility are summarized in table 2.

The requirement of the female rat for alpha tocopheryl acetate

The female rats on the E-low ration showed a plateau in the weight curve at an average age of 4 months. The rats receiving the three levels of the tocopherol grew as well as did their sisters reared on the natural food ration (graph 2). The animals receiving 0.75 mg. were slightly superior (although not significantly so) to those receiving 0.1 and 0.25 mg.

Each individual female rat employed in the reproductive studies was bred with normal males until three litters were secured. The first breeding was initiated at the first pro-oestrus after 90 days. All fifteen of the vitamin E deprived females underwent typical resorption gestations. The second

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[5] Table 2 shows that two rats in the group retained fertility until the sixteenth month.

[6] Curious and unaccountable variations were noted as regards the exact histological condition of the testes in the 0.25-mg. group, for in some instances there were a few normal testicular tubules surrounded by degenerated tubules (fig. 4). At this age, as is perhaps unnecessary to state, complete degeneration of the tubules was found in the E-low and 0.10-mg. groups.

TABLE 4

Summary of organ weights — (females, age 19 months).

GROUP	NO OF RATS	BODY WEIGHT	UTERUS & OVIDUCTS	OVARIES	ADRENALS	THYROIDS	LIVER	KIDNEYS	HEART
		gm.	gm.	mg	mg	mg.	gm.	gm.	gm.
E-Low[1]	3	253	1.41	44	83	30	9.74	2.21	1.08
		214-295	0.65-2.61	33-59	70-104	17-43	8.60-11.91	1.97-2.63	...
0.1 mg.[1] α-tocopheryl acetate	5	326	0.93	59	71	31	11.62	2.09	1.00
		275-398	0.76-1.06	48-76	51-91	20-38	8.94-14.07	1.83-2.38	0.86-1.14
0.25 mg.[1] α-tocopheryl acetat	5	346	0.97	69	81	35	11.40	2.48	1.05
		293-434	0.69-1.18	33-103	60-101	28-52	9.40-14.06	1.87-2.82	0.90-1.13
0.75 mg.[1] α-tocopheryl acetat	5	369	1.05	56	78	35	12.48	2.43	1.14
		345-423	0.55-151	27-76	68-91	13-45	11.17-13.78	2.16-2.75	1.05-1.30
Diet I + Lettuce[3]	5	320	1.29	59	66	36	12.07	2.19	1.09
		265-388	0.75-2.05	32-93	49-109	21-50	9.26-17.24	1.74-3.45	0.82-1.41
E-Low[2]	5	266	1.31	35	76	27	10.21	2.12	1.17
		212-339	0.83-1.53	26-59	60-92	17-37	8.50-12.69	1.62-2.67	0.98-1.44
0.10 mg.[2] α-tocopheryl acetat	5	359	1.09	52	66	32	11.65	2.38	1.06
		331-401	0.67-124	30-85	48-89	26-46	9.77-13.75	2.04-2.68	0.93-1.20
0.25 mg.[2] α-tocopheryl acetat	5	359	0.85	58	71	35	11.07	2.46	1.13
		279-419	0.60-1.27	28-114	55-91	27-42	9.84-11.64	2.30-2.64	1.07-1.19
0.75 mg.[2] α-tocopheryl acetat	5	355	0.79	62	63	22	11.26	2.43	1.15
		331-388	0.56-1.11	35-100	59-75	16-29	10.00-12.26	2.31-2.50	1.12-1.19
Diet I +[2] Lettuce	5	307	0.61	55	59	39	9.85	2.08	1.01
		271-335	0.47-0.76	30-116	45-73	27-45	8.42-11.30	1.80-2.41	0.91-1.14

[1] Virgin females   [2] Breeding females

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breeding during the seventh and eighth months, and the third breeding in the tenth and eleventh months yielded similar results.

The animals showing the implantation sign littered on all levels of the tocopherol (table 5). The average size of the litters and the average weight of the young were essentially the same for all treated groups as for the stock ration.
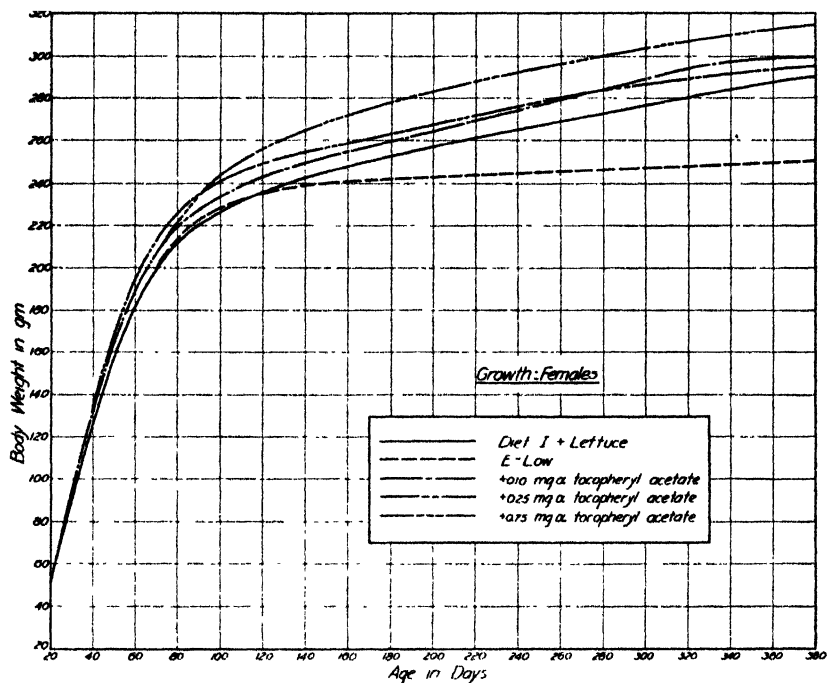
Graph 2

It is of interest to note that the typical dark brown discoloration of the uterus reported by Martin and Moore ('36) was observed in all animals in the E low group and in those receiving 0.1 and 0.25 mg. of the tocopherol (autopsied at 19 months), but not in those receiving 0.75 mg.

As was the case with the males, the striated musculature was found to be normal at autopsy on the nineteenth month in all groups receiving tocopherol, whereas the females in the

TABLE 5

Summary of reproductive behavior of females and condition of young.

LITTER	GROUP	NUMBER OF RATS	NUMBER OF LITTERS	TOTAL NUMBER OF LIVING YOUNG	AVERAGE WEIGHT AT BIRTH	% SURVIVING ON DAY 21	AVERAGE WEIGHT ON DAY 21	% SURVIVING ON DAY 30	AVERAGE WEIGHT ON DAY 30	AVERAGE DAY PARALYSIS NOTED	% YOUNG PARALYSED	% YOUNG RECOVERED ON DAY 30
					gm.		gm.		gm.			
First	E-Low	15	0									
	0.1 mg.	15	15	133	5.7	75	46	43	62	Day 20	100	23
	0.25 mg.	15	15	123	5.8	70	51	67	85	Day 20	24	82
	0.75 mg.	15	14	115	5.8	84	53	84	92	None	None	
	Diet 1	14	14	130	5.9	89	47	89	83	None	None	
Second	E-Low	13	0									
	0.1 mg.	14	12	110	5.9	73	46	48	53	Day 20	100	10
	0.25 mg.	14	14	122	5.8	82	54	80	85	Day 21	54	58
	0.75 mg.	14	13	114	6.2	93	54	92	88	None	None	
	Diet I	14	11	87	6.3	89	48	93	88	None	None	
Third	E-Low	8	0									
	0.1 mg.	12	9	50	5.9	39	43	3	45	Day 20	100	None
	0.25 mg.	14	10	64	5.4	75	47	48	55	Day 21	100	None
	0.75 mg.	14	10	82	5.6	82	52	80	88	Day 23	30	100
	Diet I	14	8	58	5.9	75	56	73	88	None	None	

E low group showed motor impairment and histological lesions in the striated musculature.

The requirement of suckling young for alpha tocopherol

The mothers in the several groups were allowed to suckle six young. At the onset of lactation these mothers and the young in these groups were indistinguishable. All were vigorous and of normal size. As regards the first litters from mothers receiving 0.1 mg. tocopherol, the suckling young appeared normal until the twentieth day when the characteristic muscular dystrophy known in E deficiency appeared. The young from mothers receiving this level of tocopherol were uniformly dystrophic.[7] Approximately one half of the young

[7] This particular intake of alpha tocopherol seemed ideal for the production of this clinical picture. The condition has previously been produced experimentally by allowing females exhibiting first litter fertility to suckle their young or by the administration of sufficient alpha tocopherol or another source of vitamin E to a pregnant female of previously established E-avitaminosis. Under such experimental conditions all young are not dystrophic.

survived 30 days. Twenty-three per cent of the animals that had been dystrophic prior to 30 days had recovered by that time. The weights of such recovered animals were markedly subnormal despite the fact that the food was accessible, i.e., placed in a low dish on the floor of the cage.

The 0.25-mg. level of alpha tocopheryl acetate did not afford complete protection against the dystrophy as 24% of the young from these mothers were paralyzed; however, the degree of impairment was greatly reduced at this level and 82% showed complete clinical recovery by day 30. The weights for the young in this group were normal.

The young reared by mothers on the highest level of tocopherol (0.75 mg.) were normal in all respects and surpassed their stock ration cousins in weight and general well-being.

A second breeding resulted in almost identical findings; note should be taken however, of an increased incidence of dystrophy in the 0.25-mg. group. When discussing the males, emphasis was given the fact that inadequacy of the lower levels of tocopherol appeared relatively late. The same phenomenon was seen as regards muscular dystrophy in the suckling young.

As regards the third breeding, on the lowest (0.10 mg.) level of the tocopherol only 3% survived 30 days and 100% of the young in the 0.25-mg. group were paralyzed; all animals living at 30 days showed some evidence of dystrophy. Some slight muscular impairment was noted in the young of mothers on the highest level (0.75 mg.) of alpha tocopheryl acetate; however, all had recovered by the thirtieth day.

DISCUSSION

These studies indicate that with the basal diet here employed the daily requirement of the rat for alpha tocopheryl acetate for normal growth and normality of the striated musculature is approximately 0.1 mg., although the actual value may be somewhat less. The daily requirement for normal reproduction in the male is somewhat below 0.75 mg. For the prevention of muscular dystrophy in suckling young born

after the tenth month of life, more than 0.75 mg. of tocopherol daily is required (table 5, third litters). These figures are many times higher than the optimal level suggested by Mason, but it must be pointed out that Mason's experiments were of short duration and that his animals were sacrificed at the age at which our breeding tests were initiated. The basal diets were not identical. Mason did not study the tocopherol requirement during lactation for the prevention of muscular dystrophy in the suckling young.

The quantity of vitamin E received by the mother does not materially affect either the quantity or quality of milk produced save in the one respect as to its E content, for the young prior to the onset of paralysis are usually normal in size.

SUMMARY

1. Growth and reproduction were studied for 16 months in male and female rats maintained from weaning on a vitamin E low diet (Diet 427), and supplemented with three levels of alpha tocopheryl acetate; the levels were 0.10 mg., 0.25 mg., and 0.75 mg. six times weekly.

2. The tocopherol requirement for normality of the striated musculature and for growth in the case of both sexes is less than 0.10 mg. daily.

3. As regards the males, the 0.10-mg. level was inadequate for the preservation of fertility beyond the fifth month. The 0.25-mg. level was adequate for normal reproduction during approximately the first 9 months of life, but proved inadequate for the subsequent maintenance of fertility. The highest level of tocopherol (0.75 mg. daily) maintained normal testes and fertility during the entire experimental period of 16 months.

4. As regards the females, those receiving 0.10 mg. daily were able to give birth to living young even in their third gestation which was completed between the eleventh and twelfth months of age. By the eighteenth month orange-brown pigmentation of the uterus supervened in all animals receiving 0.10 mg. and 0.25 mg. daily dosage but was absent in the group receiving 0.75 mg.

5. As regards the suckling young, in contrast to the adult female, all those born from mothers receiving 0.10 mg. daily dosage exhibited muscular dystrophy with a high mortality rate in the third week of life. Those born from mothers receiving 0.25 mg. daily dosage exhibited a lessened incidence of dystrophy with a lowered mortality rate until the third breeding when all young were paralyzed and died as was the case in litters from mothers receiving 0.10 mg. All young from mothers receiving 0.75 mg. daily dosage were normal in the first two breedings, but in the third gestation there was a slight incidence of dystrophy with complete recovery by the thirtieth day of life.

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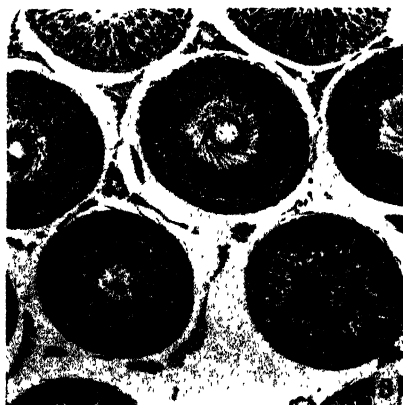
1   Typical area from cross section of the testis of rat 17 months old, reared and maintained on natural stock diet (Diet I and lettuce). × 135.

2   Typical area from cross section of testis of 17-month-old rat, reared and maintained on vitamin E-low diet (Diet 427). Complete degeneration of the seminiferous epithelium is shown. This rat was sterile after 4½ months of age. × 135.

3   Typical area from cross section of testis of 17-month-old rat reared and maintained on vitamin E-low diet but supplemented with 0.75 mg. a tocopheryl acetate six times weekly. × 135.

4   Cross section of testis of rat 21 months old, reared and maintained on vitamin E-low diet supplemented with 0.25 mg. a-tocopheryl acetate six times weekly. × 135.

THE INFLUENCE OF NUTRITION DURING PREGNANCY UPON THE CONDITION OF THE INFANT AT BIRTH

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TWO FIGURES

Nutrition has not occupied a place of major importance as a part of prenatal care. The obstetrician's primary interest is not so much the health and development of the unborn infant as the health of the mother during pregnancy. Interest in diet during the gestation period, except in so far as it relates to excessive weight gain of the mother, has been relatively slow to develop, because the medical profession has not been convinced that diet during pregnancy occupies a place of great importance in regard to either the mother or her offspring. Heredity, nutrition, activity and disease are the principal factors which determine the course of physical growth and development. The relative importance of each has not been established, but it is becoming increasingly clear that "from conception to death, health is primarily dependent upon food and nutrition" (Macy, '42). It is also recognized (Adair, '32) that although in the earliest part of pregnancy the ovum carries some nutrient material within itself, it is largely dependent upon the mother for the material from which it is to grow and develop.

The need for better nutrition during the period of pregnancy has been repeatedly emphasized (Garry and Stiven, '36; Dieckmann and Swanson, '39; Burke, '41). While no attempt will be made here to review the literature completely, by way of historical background, attention is called to a few of the many illustrations of fetal damage resulting from prenatal dietary deficiency of various types which are to be found in the records of animal experimentation (Hart, Steenbock and Humphrey, '20; Smith, '17; Hale, '37; Warkany and Nelson, '42). Interesting evidence of damage to the human fetus also, due to inadequate maternal nutrition during pregnancy, has been reported (Maxwell, Pi, Lin and Kuo, '39; Toverud, '38; Burke, '40; Editorial, '32; Teel, Burke and Draper, '38). The metabolism studies of Macy and her coworkers ('42) and of Coons, Schiefelbusch, Marshall and Coons ('35), carried out on women during the reproductive cycle, contributed to our knowledge of the nutritional requirements of women in this period. The recent works of Ebbs, Tisdall and Scott ('41) evaluates the diet of the mother during pregnancy in relation to her condition during the course of pregnancy, labor, delivery, and the postpartum period, as well as to the condition of the infant at birth and within the early months of life. Recent English studies (Interim Report, '42) report the effect of improved nutrition of expectant mothers on maternal and infant morbidity and mortality by the use of concentrates.

GROUP STUDIED AND METHODS

The study of the influence of diet during pregnancy upon fetal growth and development, as well as upon the course of pregnancy, labor, delivery, and the puerperium, is a part of the research program on the growth and development of the well child, undertaken by the Department of Child Hygiene of the Harvard School of Public Health. The work was begun in 1930, and the program enlarged to include the establishment of a Center for Research in Child Health and Development in

1932.[1] A detailed description of the work at the Center is given in an early publication (Stuart and Staff, '39).

Three-hundred and twenty-four infants have been enrolled at the Center. In this paper observations on 216 women and their infants are presented. In each of the 216 cases the mother has been enrolled at the Center for the first time, and the resulting infant is the oldest sibling of that family in the study. Pregnancies resulting in younger siblings and in twins have been omitted but will be presented in a later paper. This main group of mothers and their infants represents a sampling of cases uninfluenced previous to the pregnancy under study.

The women were selected from the prenatal clinics of the Boston Lying-in Hospital and were cared for in the early years of the study by the staff of that hospital. Since 1935 they have been under the care of obstetricians attached to the staff of the Center, who have also been members of the hospital staff. These women were seen at least monthly through the seventh month of pregnancy, every 2 weeks during the eighth month, and then weekly, unless it became necessary to see them more frequently because of complications.

The pediatric ratings describing the condition of each infant at birth and within the first 2 weeks of life are based upon the obstetrician's evaluation of the infant's condition at birth, together with a routine physical examination of each infant by a pediatrician from the staff of the Center within 48 hours of birth (the majority within 24 hours of birth), and another routine physical examination by a staff pediatrician before discharge from the hospital, usually on the fourteenth day of life. Careful notes were made of the progress of each infant while in the nursery, and all infants whose neonatal course was not entirely satisfactory were visited frequently during that period by one of the Center's pediatricians.

The nutrition data are based upon detailed dietary histories obtained at the time of the mother's first visit to the prenatal clinic and at as many subsequent visits as the individual case required, at least once each trimester. These nutrition histories include a complete record of the food intake for the 24-hour period preceding the clinic visit, data about the woman's normal food habits and any changes which have been made in these during the course of pregnancy. In addition, at regular intervals during the course of pregnancy each patient kept a consecutive 3-day record of food consumed. Information in regard to food likes and dislikes, amounts of various foods purchased, amount of money spent for food in relation to total weekly income and number of persons in the family, and other related data, have been recorded. The average consumption of important foods, such as milk, eggs, meat, fish, poultry, liver, whole grain cereals and breads, vegetables, fruit, etc., have been carefully checked. An investigation was also made of the amount of highly refined carbohydrate and fat-rich foods eaten, in an attempt to determine to what extent foods of these types were replacing foods rich in proteins, minerals, and vitamins. Such a method of cross-checking important data has made a valuable contribution in greater accuracy of the final dietary ratings. The forms used in taking these nutrition histories, and further details as to the method, are given in a monograph (Stuart and Staff, '39).

The method used in evaluating these diets is also described in detail in another publication (Burke and Stuart, '38). In evaluating these diets for the last two trimesters of pregnancy, comparison has been made with an arbitrary set of standards (table 1) selected as "optimal," which approximate the values later recommended by the Food and Nutrition Board of the National Research Council.

No nutrient was called "excellent" unless it met or exceeded this "optimal" standard. Each nutritional essential was rated "excellent", "good", "fair", "poor", or "very poor", according to the range within which it appeared to fall. If the variations in the intake of any nutrient made the exact rating

TABLE 1

Nutritional standards used in rating dietary intakes during pregnancy (4th through 9th months)

NUTRITIONAL ESSENTIALS	EXCELLENT	GOOD	FAIR	POOR	VERY POOR
Protein, gm.	85 or more	70–84	55–69	45–54	Under 45
Calcium, gm.	1.5	Under 1.5–1.0	Under 1.0–.75	Under .75–.6	Under .6
Phosphorus, gm.	2.0	The same rating was given phosphorus in each case as was assigned to protein.			
Iron, mg.	20	Under 20–16	Under 16–12	Under 12–10	Under 10
Vitamin A, I.U.	8000	Under 8000 to 6000	Under 6000 to 4000	Under 4000 to 2000	Under 2000
Thiamine, mg.	2.0	Under 2.0–1.0	Under 1.0 to above .5	.5–.3	.3
Riboflavin, mg.	2.5	Under 2.5–2.0	Under 2.0–1.5	Under 1.5–1.2	Under 1.2
Niacin, mg.	18	No attempt was made to rate niacin.			
Ascorbic acid, mg.	100	Under 100–75	Under 75–50	Under 50–35	Under 35
Vitamin D, I.U.	400–800	These were used merely as "excellent" with added D and "poor" without added D.			

of the average intake doubtful, a rating has been assigned to indicate this, for example, "fair to poor". Therefore, each dietary rating is an average estimate and falls approximately within a range represented by the terminology "excellent", "good", "fair", etc., as shown in table 1. A daily caloric intake above 2800 has been termed "excessive" and below 2200 Calories "inadequate" in all cases. Judgment in regard to the individual case has to a large extent determined the rating assigned, if the caloric intake was between these two extremes. A closer analysis of data obtained by the nutrition history method is unwarranted, and more exact calculation of diets is justified only in the case of weighed samples.

The mean general dietary rating used as representative of each diet during the major portion of pregnancy was obtained by averaging numerical values given to the ratings assigned to the nutritional essentials: "excellent" $= 4$, "good" $= 3$, "fair" $= 2$, "poor" $= 1$, and "very poor" $= 0$. This method of obtaining the mean rating assigned to the average dietary intake of each woman during pregnancy has resulted in more accurate evaluations when used in comparative case studies, as the relative relationship of each dietary rating to every other is exact. This has greatly facilitated the statistical handling of the nutritional data.

Of the 216 cases studied, only 14% of the women consumed diets during pregnancy (fourth through ninth month) which could be considered "excellent" or "good" according to the nutritional standards given in table 1. The diets consumed by 17% of the women were "fair to good", 29% had "fair" diets, 23% "fair to poor" diets, and 17% had diets for this period which were "poor to very poor". This means that at least 40% of the women in this group were definitely malnourished, according to these dietary standards, during a period when the fetus undergoes very rapid growth and development, and that another 29% had only mediocre diets for this period.

The parents of these infants are largely of North European stock, as is shown in table 2, and represent, from an economic standpoint, the average "middle class" American family.

The economic depression of the early nineteen-thirties intervened to move a number of families to a lower economic level. A few cases from the upper economic brackets are included, usually individuals close to the study staff who provided an opportunity for the collection of unusually reliable data. Table 3 shows the relationship of income to number of dependents within each family during the pregnancy under study.

The ages of the parents at the time of the birth of these infants are given in table 4.

TABLE 2

Racial background of parents.

RACIAL BACKGROUND	NUMBER OF CASES	PER CENT
Irish	46	21.3
"American" or "Canadian"	18	8.3
French-Canadian, French, Scotch, English, Irish	23	10.6
Scotch, English, Irish	56	25.9
Scandinavian, Finnish, German, Dutch, American, etc.	48	22.2
TOTAL OF COMBINED NORTH EUROPEAN STOCK	191	88.3
Italian or Part Italian	9	4.2
Jewish or Part Jewish	9	4.2
Others (Albanian, Polish, Syrian, etc.)	7	3.3
TOTAL OTHER THAN NORTH EUROPEAN STOCK	25	11.7
Total	216	100.0

The medical data concerning the mothers, together with the findings in regard to the influence of diet during pregnancy upon the course of pregnancy, labor, delivery and the postpartum period, are given in a recent publication (Burke, Beal, Kirkwood, and Stuart, 1943).

The pregnancies of these women terminated in an almost equal number of male and female infants, 107 male and 108 female infants, and one stillborn infant whose sex was undetermined. There were 200 full term, 9 premature,[2] 5 stillborn infants, and 2 infants who died within a few hours of birth.

[2] Three of these infants were included because one specialist in the group was particularly interested in the study of premature infants.

TABLE 3

Relation of income to number of dependents in family during prenatal period.

INCOME PER WEEK	SIZE OF FAMILY			NUMBER OF CASES	PER CENT
	2 Adults	0 Children	17		
$19.00 or less	2 Adults	1 Child	3	21	9.7
	2 Adults	2 Children	1		
$20.00–$24.00	2 Adults	0 Children	22	30	13.9
	2 Adults	1 Child	6		
	2 Adults	2 Children	1		
	2 Adults	6 Children	1		
	2 Adults	0 Children	40		
$25.00–$29.00	2 Adults	1 Child	9	52	24.1
	2 Adults	2 Children	3		
	2 Adults	0 Children	58		
$30.00–$39.00	2 Adults	1 Child	15	76	35.2
	2 Adults	2 Children	2		
	2 Adults	3 Children	1		
	2 Adults	0 Children	18		
$40.00–$49.00	2 Adults	1 Child	3	23	10.6
	2 Adults	2 Children	2		
	2 Adults	0 Children	6		
$50.00 or more	2 Adults	1 Child	4	12	5.6
	2 Adults	2 Children	2		
No data	2 Adults	0 Children	2	2	0.9
Total number of families				216	100.0

TABLE 4

Age of parents at infants' birth.

AGE DISTRIBUTION	MOTHERS		FATHERS	
	No. cases	%	*No. cases*	%
20 yrs. or under	24	11.1	5	2.3
21–25 yrs.	79	36.6	63	29.1
26–30 yrs.	70	32.4	76	35.2
31–35 yrs.	33	15.3	52	24.1
36 yrs. or over	10	4.6	20	9.3
Total	216	100.0	216	100.0

RESULTS AND DISCUSSION

In considering any possible effects of nutrition during pregnancy on infant mortality and morbidity, the diet of the mother during pregnancy was studied in relation to the condition of the infant at birth and during the first 2 weeks of life.[3] All of the 216 infants against whom the examining pediatricians had found no physical count of any kind, either at birth or within the first 2 weeks of life, were selected as the "superior" infants of the group. There are twenty-three in this group. The birth weights and lengths of these infants, together with the mean general ratings assigned to the mothers' diets during pregnancy (fourth through ninth months) are given in table 5.

In contrast, all infants at the opposite extreme of the group in pediatric rating were next considered. The "poorest" infants included all who were stillborn, died within a few hours or days of life, had marked congenital malformations at birth, were premature (under 5 lbs. at birth), or were "functionally immature." "Functionally immature" means that the physical development or reactions of the infant were considered below normal by the pediatrician, and does not apply to weight and length alone. There were thirty-three infants in the poorest group, and the data concerning them are presented in table 6.

The remaining infants, of whom there are 160, were subdivided into two groups. All infants against whom the pediatric rating showed only one or two minor physical counts were considered in "good" physical condition. There are eighty-four infants in this classification. All infants not in one of the three already defined classifications were placed in one group termed "fair" (some of these were considered to be in fair to poor physical condition, others were definitely in a fair to good classification according to the pediatric findings). There are seventy-six "fair" infants. In interpreting these

[3] It should be remembered in studying the relationships obtained that the ratings used in each field (pediatric, obstetric, nutritional, anthropometric, etc.) have been made by the specialist in charge of each field of endeavor, and the results assembled to determine possible associations of statistical significance.

groupings, it should be remembered that those infants termed "good" are relatively near the superior infants in physical condition, while the group called "fair" represent a much wider range. The relationship existing between the physical condition of the infant and the mother's diet during pregnancy

TABLE 5

Mean ratings of prenatal diets, birth weights, and birth lengths of "superior infants".[1]

CASE NO.	CONDITION OF INFANT	WEIGHT		LENGTH	PRENATAL DIET MEAN RATING
		lbs.	ozs.	cm.	
5		7	0	50.5	Fair
16		6	12	47.5	Excellent
20		7	0	49.2	Good
24		8	2	52.1	Fair to poor
37		10	3	53.1	Excellent
43		8	14	53.3	Fair to good
49		8	14	51.6	Good
52	All infants are "Superior"	9	2	51.8	Good
91		8	9	51.4	Excellent
133		7	11	51.0	Good
142		8	5	50.8	Good (to fair)
154		6	10	50.3	Poor (to very poor)
189		11	7	53.5	Good
192		8	8	50.7	Fair
198		6	15	47.6 (7 days)	Fair
207		7	4	50.6	Fair
210		7	15	50.6	Fair
220		7	13	51.2	Good to excellent
240		7	14	51.2	Fair
248		7	12	51.4	Fair
265		7	11	51.4 (5 days)	Good
290		8	8	49.9	Good
323		8	5	52.1 (5 days)	Excellent

[1] All infants against whom there was no physical count of any kind recorded either at birth or within the first 2 weeks of life.

is shown graphically for the entire 216 cases in figure 1 (cases selected on the basis of pediatric ratings).

Similar correlations are found when the cases are selected on the basis of the rating assigned to the diet of the mother during pregnancy. This relationship is shown graphically in figure 2 (cases selected on the basis of prenatal dietary rat-

TABLE 6

*Mean ratings of prenatal diets, birth weights and
birth lengths of "poorest" infants.*[1]

CASE NO.	CONDITION OF INFANT	WEIGHT		LENGTH	PRENATAL DIET MEAN RATING
		lbs.	ozs.	cm.	
17	Stillborn	5	12	(54.6)[2]	Very poor
36	Stillborn	6	15	(50.8)[2]	Very poor
48	Stillborn. (Macerated)	4	12	.....	Very poor
110	Stillborn	7	8	.....	Poor (to very poor)
237	Stillborn. Macerated. Multiple congenital anomalies.	Del. 3 mos. premature		.....	Very poor
199	Died at birth. Erythroblastosis. Congenital urethral stenosis.	8	15	(53.3)[2]	Poor to very poor
205	Died in 2 hours. Multiple congenital anomalies.	6	12	48.5	Fair
8	Died 3rd day (bronchitis & broncho-pneumonia)	6 (2 days)	1	(52.1)[2]	Poor (to very poor)
235	Died at 5 mos. Congenital malformation heart	3	4	... .	Very poor
81	Congenital heart	8	11	52.6	Excellent
127	Congenital heart	7	0	49.4	Poor to very poor
108	Mild erythroblastosis	7	2	50.6	Very poor 7 mo. then good to excellent
116	Bifid uvula	8	9	52.3	Fair (to poor)
58	Congenital mobile funnel chest	8	14	51.6	Fair
120	Arrested hydrocephalus Premature	3	12	40.6 (14 days)	Poor to very poor
231	Cleft palate. (Feeble-minded)	8	0	48.9	Poor to very poor
191	Inguinal hernia. Premature	3	6	44.5 (26 days)	Fair
122	Congenital cataracts. Premature (mentally retarded at 1 yr.)	3	5	41.9 (14 days)	Poor to very poor
158	Premature	3	14	43.2 (30 days)	Poor to very poor
204	Premature	4	11	47.0 (11 days)	Poor to very poor
206	Premature	3	12	44.5 (30 days)	Poor to very poor
211	Premature	5	0	45.7 (5 days)	Poor to very poor
281	Premature	4	13	45.7 (14 days)	Poor to very poor
69	Functionally immature	5	14	48.9 (13 days)	Very poor
42	Functionally immature	6	0	46.6	Poor to very poor
67	Functionally immature	5	6	47.4	Fair
76	Functionally immature	5	4	48.5	Poor to very poor
93	Functionally immature	6	6	50.6	(Fair to) poor
109	Functionally immature	6	4	48.4	Poor (to very poor)
125	Functionally immature	5	13	47.0 (6 days)	Poor (to very poor)
196	Functionally immature	5	8	45.6	Poor to very poor
213	Functionally immature	6	12	48.2	Fair
238	Functionally immature	6	12	48.0	Fair (?not utilized)

[1] All infants who were stillborn, died within a few hours or days of life, had a marked congenital malformation at birth, were premature (under 5 lbs. at birth) or were "functionally immature."

[2] Not measured by our staff; taken from hospital records.

ings). Table 7 gives the birth weights and lengths of the infants grouped according to the pediatric rating and according to the rating assigned to the mother's diet during pregnancy.
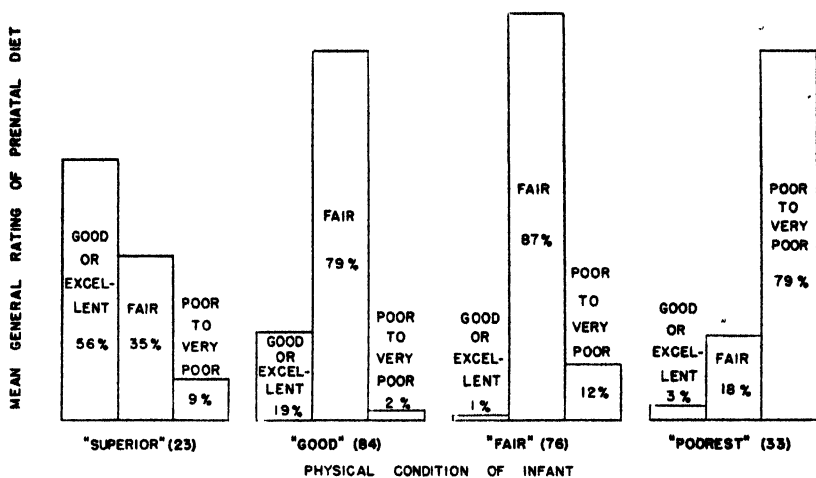
Fig. 1   Relationship of the condition of infant at birth and within first 2 weeks of life to mother's diet during pregnancy. (Cases selected on the basis of pediatric ratings of infants, 216 cases).
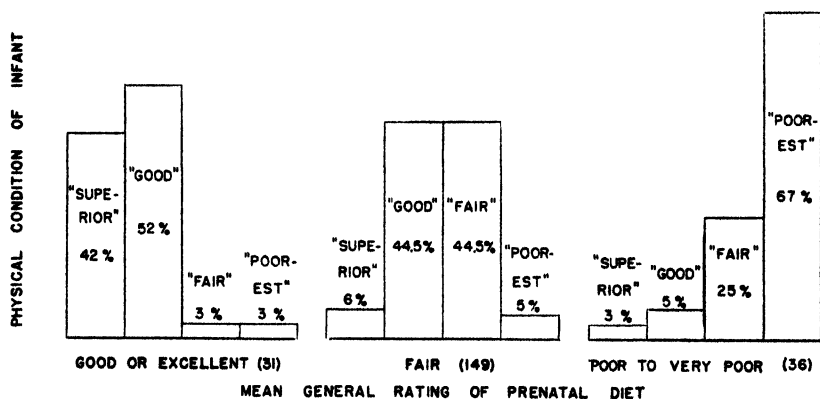
Fig. 2   Relationship of prenatal nutrition to the physical condition of the infant at birth and within first 2 weeks of life. (Cases selected on the basis of mean rating assigned to mothers' diets during pregnancy, 216 cases).

TABLE 7

Birth weights and lengths of infants grouped according to pediatric rating and according to prenatal dietary rating.

	PEDIATRIC RATING				PRENATAL DIETARY RATING		
	"Superior"	"Good"	"Fair"	"Poorest"	Excellent or Good	Fair	Poor to Very Poor
Birth weight lbs., oz.							
Average	8–2	7–12	7–2	5–15	8–8	7–7	5–13
Range	6–10 to 11–7	5–8 to 10–12	5–4 to 9–3	3–4 to 8–15	6–12 to 11–7	3–6 to 9–3	3–4 to 8–15
Birth length cm.							
Average	50.8	50.6	49.5	47.2	51.8	50.0	47.2
Range	47.0 to 53.3	46.2 to 54.4	45.0 to 54.4	40.6 to 52.1	46.9 to 54.6	45.0 to 54.4	40.6 to 52.7

CONCLUSIONS

1. A statistically significant relationship has been shown between the diet of the mother during pregnancy and the condition of her infant at birth and within the first 2 weeks of life.

2. If the diet of the mother during pregnancy is poor to very poor, she will undoubtedly have an infant whose physical condition will be poor. In the 216 cases considered in this study, all stillborn infants, all infants who died within a few days of birth except one, most infants who had marked congenital defects, all premature, and all functionally immature infants were born to mothers whose diets during pregnancy were very inadequate.

3. If the mother's diet during pregnancy is good or excellent, her infant will in all probability be in good or excellent physical condition. It may, however, happen rarely that a mother whose diet during pregnancy is good or excellent will give birth to an infant in poor physical condition (1 out of 216 cases in this study).

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EFFECTS OF CECECTOMY, SUCCINYLSULFATHIAZOLE, AND p-AMINOBENZOIC ACID ON VITAMIN K SYNTHESIS IN THE INTESTINAL TRACT OF RATS[1]

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The synthesis of certain vitamins in the intestinal tracts of man and animals has been recognized for many years (Anonymous, '43). Guerrant, Dutcher and Tomey ('35), Griffith ('35), Tange ('39), and others, reported the effect of the type of carbohydrate in the diet in relation to intestinal synthesis of B vitamins. Nielsen, Shull and Peterson ('42) found that intestinal synthesis of biotin varied with different rations and was particularly low when the diet contained inadequate amounts of riboflavin. However, progress in the study of vitamin synthesis by intestinal microorganisms was greatly limited until certain sulfonamides with satisfactory bacteriostatic effects were introduced (Black, McKibbin and Elvehjem, '41; Mackenzie, Mackenzie and McCollum, '41; Welch, '42; Poth and Ross, '43). The availability of succinylsulfathiazole (sulfasuxidine), which seems in certain cases to be more suitable than sulfanilylguanidine (sulfaguanidine) (Welch, '42), has extended the possibilities for investigation along these lines.

The antagonizing effect of p-aminobenzoic acid on the inhibition of growth caused by sulfaguanidine was demonstrated

by Black, McKibbin and Elvehjem ('41), Mackenzie, Mackenzie and McCollum ('41) and others. Although it was first believed (Welch, '42) that p-aminobenzoic acid does not inhibit the bacteriostatic effect of sulfasuxidine, it has been recently reported that the compound does partially counteract the inhibiting effect of sulfasuxidine on growth (Welch and Wright, '43; Neumann, Krider and Day, '43). No reports have been made concerning the effect of p-aminobenzoic acid on the hypoprothrombinemia produced, under certain conditions, by feeding sulfasuxidine.

Investigation of the possible role of the cecum in vitamin synthesis was stimulated by Guerrant et al. ('35) who noted that the cecum was unusually large in rats fed carbohydrates which promote relatively abundant synthesis of certain B complex vitamins. Moreover, such ceca contained large numbers of live yeast cells. Griffith ('35) cecectomized over 200 weanling rats and determined the effect on growth when the diet was deficient in one or more groups of water soluble vitamins. Since coprophagy improved the condition of the operated animals it was concluded that vitamin synthesis occurs in other parts of the alimentary tract. Innes and Nicolaysen ('37) cecectomized about 100 young rats in order to study the effect of the cecum on phosphorus utilization from phytin. Taylor, Pennington and Thacker ('42) found that cecectomized rats fed a good stock diet were apparently unaffected but when the quality of the ration was impaired by dilution, largely with sucrose, the operated animals became markedly debilitated while the unoperated controls remained in good health. Their studies suggested that "where the diet supports the right intestinal flora, the cecum contributes to the rat's supply of several of the B vitamins." These investigations indicate the importance of the cecum in the synthesis of vitamins and the possible value of cecectomized animals in the study of such problems.

Several investigations have shown the synthesis of vitamin K by the intestinal flora of rats (Dam et al., '37; Greaves, '39) and of ruminants (McElroy and Goss, '40). Black, Overman,

Elvehjem and Link ('42) and Welch and Wright ('43) seem to be the only workers who have reported the effect of sulfonamides on this synthesis. Lockhart, Sherman and Harris ('42) noted extreme hypoprothrombinemia in rats fed dihydroxystearic acid. The deficiency syndrome could be cured or prevented by 2-methyl-1, 4-naphthoquinone.

The study to be reported here was carried out primarily to determine (1) whether the cecum is a significant source of vitamin K to the rat and (2) to what extent sulfasuxidine and p-aminobenzoic acid affect the synthesis of this vitamin in the intestinal tract.

EXPERIMENTAL

Young, piebald rats of the McCollum strain were used. In all experiments except those involving cecectomy the starting weight was 40 to 55 gm. Cecectomized animals weighed 60 to 80 gm. at the time of the operation. The animals were divided as equally as possible on the basis of litter membership, sex and weight. Although they were kept on screens, precaution was taken to keep the cages clean in order to reduce coprophagy to a mininum.

For prothrombin time determinations blood was obtained by cardiac puncture under ether anesthesia, except in the early part of the study when it was secured from the exposed inferior vena cava of the animals which were subsequently autopsied. In the latter cases only one sample, of course, was obtained from each animal. The blood, 1.8 ml., was drawn into a clean syringe containing 0.2 ml. of 0.1 M sodium oxalate and then carefully discharged into a clean 15 ml. centrifuge tube. The prothrombin time was determined on diluted plasma by a modification of Quick's method (Pohle and Stewart, '39). All the prothrombin times reported in this study are in terms of 12.5% plasma. In general, blood was taken for prothrombin determinations biweekly. Uniform procedures were applied throughout this study.

In analyzing the data on plasma prothrombin time all values above 50 sec. were interpreted as proof of definite hypopro-

thrombinemia. All values below 50 sec. were regarded as essentially normal. Overman et al. ('42), using approximately the same method as we have employed, found that the range of prothrombin time for normal rats on stock diets is about 36 to 45 sec. Our range of values for apparently normal rats is about 32 to 42 sec. Consequently it would seem that our criterion of hypoprothrombinemia is a conservative and valid one.

The experimental diets used are indicated in table 1. Vitamin mixture 1 contained thiamine, 10 mg.; pyridoxine, 10 mg.; riboflavin, 20 mg.; nicotinic acid, 200 mg.; calcium pantothenate, 200 mg.; inositol, 400 mg.; and glucose, 9.17 gm. Vitamin mixture 2 was the same except that it contained 200 mg. p-aminobenzoic acid and a correspondingly smaller amount of glucose. The dry vitamin-sugar mixture was made up in sufficient amounts to last several weeks and was kept in the refrigerator. Owing to the relatively large amount of sugar contained in the mixture the designated quantity of vitamins per kilogram of diet could be readily weighed on the scales used for the other components of the diets.

Salts 2 contained $NaHCO_3 \cdot H_2O$, 95; $CaHPO_4 \cdot 2H_2O$, 215; KCl, 152; $MgSO_4 \cdot 7H_2O$, 98; iron citrate, 10; $ZnSO_4 \cdot 7H_2O$, 2.5; $MnSO_4 \cdot 2H_2O$, 2.0; $CuSO_4 \cdot 5H_2O$, 1.0; and KI, 0.5.

When it became difficult to secure sucrose, because of the war rationing, glucose was substituted, as indicated in table 1.

Cecectomy

A paramedian incision is made and the skin flaps are separated along the fascial plane to expose the muscular layer of the abdominal wall. A median incision along the linea alba exposes the abdominal viscera with the least amount of hemorrhage. The cecum with its attachments to the terminal ileum and the proximal portion of the colon is lifted out of the peritoneal cavity through the opening in the abdominal wall and the laparotomy cloth is overlapped in order to protect the peritoneal cavity. The blood vessels supplying the cecum are doubly ligated and sectioned between ligatures and the ends

TABLE 1

Percentage composition of diets.

CONSTITUENTS	1	2	3	4	5	6	7	8	9	10	11	12
							DIETS					
Sucrose	70.5	70.5	70.5	70.5	69.6	70.6	69.6	70.6				
Glucose (Cerelose)									70.0	70.0	66.0	65.0
Casein (Smaco)											18.0	18.0
Casein hydrolysate	18.0		18.0				18.0	18.0				
Brewer's yeast		18.0		18.0	18.0	18.0			18.0	18.0	7.0	7.0
Salts 2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0	4.0
Vitamin mixture 1	1.0		1.0	1.0	1.0	1.0	1.0	1.0				
Vitamin mixture 2		1.0							1.0	1.0		
Corn oil (Mazola)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cod liver oil									2.0	2.0	2.0	2.0
Percomorph liver oil	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]				
Choline chloride	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Sulfasuxidine	0.08	0.08	0.08	0.08	1.0		1.0		1.0	1.0		1.0

[1] Drops.

Notes on constituents of diets.

Sucrose: Commercial. Extracted 3 times with about 4 parts by weight of 95% alcohol at room temperature. Twelve hours per extraction.

Casein hydrolysate: A tryptic digest of casein, Amigen, kindly furnished by Mead, Johnson and Co., through the courtesy of Dr. W. M. Cox, Jr. Extracted 15 hours in continuous extractor using ether.

Brewer's yeast: Extracted with ether, similar to procedure for casein hydrolysate.

Sulfasuxidine: Generously supplied by Dr. W. A. Feirer, Sharp and Dohme, Inc.

Vitamins: Most of the synthetic vitamins were kindly furnished through the courtesy of Dr. D. F. Robertson, Merck and Co.

of the ileum and colon are ligated and excised. A purse-string suture is used to close the end of the ileum and of the colon. In order to establish continuity of the alimentary canal a side-to-side anastomosis is made between the terminal ileum and the proximal colon. Connell stitch is used for uniting the openings.

RESULTS

Effect of amino acid source on development of hypoprothrombinemia

When this study was started some uncertainty existed concerning the construction of satisfactory experimental diets deficient in both vitamin K and p-aminobenzoic acid. It was believed that p-aminobenzoic acid might significantly affect the bacteriostatic action of sulfasuxidine, especially if the latter was fed in small amounts (Wood, '42). Since it seemed likely that any p-aminobenzoic acid or vitamin K present in casein could be more thoroughly extracted from the hydrolyzed product, some of the earlier studies (table 2) were made using

TABLE 2

Effect of sulfasuxidine and p-aminobenzoic acid on the prothrombin time and growth of rats fed purified diets.

DIET	NO. OF RATS	AVG. WKS ON DIET	GROWTH		AVG PROTHROMBIN TIME			NO. OF RATS WITH 1 OR MORE PTS [1] OVER 50 SEC
			Max wt. (avg)	Gain (avg)	Min.	Max	Mean	
			gm.	*gm.*	*sec.*	*sec.*	*sec.*	
1 (Pab [2]—; S [3] 0.08%)	6	10	236	190	37	85	51	1 [4]
2 (Pab +; S 0.08%—)	4	13	262	213	20	85	41	1
3 (Pab +; S 0.08%)	2	9	206	106	32	35	34	0
4 (Pab —; S 0.08%)	3	9	190	84	35	36	36	0
5 (Pab —. S 1.0%)	4	9	82	41	33	105	56	2
6 (Pab —; S —)	2	9	127	89	40	42	41	0
7 (Pab —; S 1.0%)	5	4	116	47	30	70	49	4
8 (Pab —; S —)	5	4	167	101	33	62	42	1
9 (Pab +; S 1.0%)	13	5	117	65	39	51	45	4
10 (Pab —: S 1.0%)	27	5	116	60	54	83	69	16

[1] Prothrombin times.

[2] Para-aminobenzoic acid.

[3] Sulfasuxidine.

[4] Two of the six died of hemorrhages. No prothrombin time determinations were made on these two animals.

diets containing a tryptic digest of casein [3] extracted with ether.

The principal results are in table 2 (compare results from rats fed diets 1 and 2 with those on diets 4 and 3 respectively). Rats fed diets in which ether-extracted casein hydrolysate was the sole source of amino acids had higher prothrombin time values and a higher incidence of hypoprothrombinemia than those fed "vitamin free" casein.[4] No data are available with which strict comparison might be made concerning the effect of the hydrolyzed casein in diet 7. However four of the five rats fed that diet had one or more prothrombin time values above 50 sec. Even one animal fed diet 8, similar to diet 7 except that it contained no sulfasuxidine, developed a mild degree of hypoprothrombinemia. There seems to be no basis for deciding whether the difference between the two sources of amino acids might be attributed to small amounts of vitamin K or p-aminobenzoic acid in the unhydrolyzed casein. However we are convinced from the evidence on hand that "vitamin free" casein can be used satisfactorily in experimental diets to produce vitamin K deficiency in the presence of bacteriostatic agents.

Effect of different levels of sulfasuxidine on prothrombin time

Only two different levels of the sulfonamide were tested, 0.08% and 1.0%. The latter, as might be expected, caused a higher percentage of the animals to develop a prolonged prothrombin time, as shown in table 2. However, certain diets containing only 0.08% of the sulfonamide produced severe hypoprothrombinemia in some of the animals.

Bacterial counts [5]

Bacteriological studies of feces taken from the lower colon and also of the material from the cecum, both obtained at

autopsy, showed a marked reduction in the E. coli content (desoxycholate agar plates) of rats on diets 3, 4, 5, and 7 as compared with the counts from control rats on diets 6 and 8. The bacterial counts were made on rats that had been on the experimental diets for 4 to 9 weeks.

Effect of p-aminobenzoic acid on the action sulfasuxidine

Black, Overman, Elvehjem and Link ('42) found that p-aminobenzoic acid, when added to a purified diet, "counteracted growth inhibition and prolonged prothrombin time" resulting from the presence of sulfaguanidine at a 0.5% level. In a note added concerning sulfasuxidine it is stated that this sulfonamide caused changes in prothrombin time similar to those observed with sulfaguanidine and that the effects were antagonized in a similar manner by vitamin K and liver extract.

Our studies show that the prothrombin time is definitely lower when p-aminobenzoic acid is added to diets containing either 0.08% sulfasuxidine or 1.0%. Also, the incidence of definite hypoprothrombinemia is less in those receiving p-aminobenzoic acid (compare results from animals on diet 1 with those on diet 2, and diet 10 with those on diet 9, table 2). This is a striking difference.

The effect on the growth (table 2) and appearance of the animals is also clearly recognizable. Animals fed p-aminobenzoic acid gained more weight during the experimental period and were less decrepit than those deprived of it. The effect on growth was most marked after the animals had been on the diet for 3 to 4 weeks. Nearly all rats receiving 1% sulfasuxidine without p-aminobenzoic acid lost weight after 3 or 4 weeks, but those receiving an equal amount of the sulfonamide with p-aminobenzoic acid added tended to gain weight, although very slowly. For instance, only one rat in a group of thirteen fed diet 9 (p-aminobenzoic acid added) lost weight after the third or fourth week but twenty-one out of a group of twenty-seven fed diet 10 (p-aminobenzoic acid deficient) lost weight. Also, in the former group the fur was cleaner

and smoother, and the animals were less emaciated. Even the feces of those fed p-aminobenzoic acid were not as soft as those from rats not given the compound.

It seems probable that the bacteriostatic effectiveness of a sulfonamide is due to its competition with p-aminobenzoic acid for an important enzyme site on the bacterial cell (Wood, '42). In view of this it might be supposed that animals fed only 0.08% sulfasuxidine would show less growth inhibition than those fed 1.0% since the content of p-aminobenzoic acid was the same in all diets to which it was added. Our data are not sufficient to clarify this point but they do indicate that growth inhibition is greater in those fed the higher level of the sulfonamide (table 2).

Effects of cecectomy

Four main groups of animals on the basal vitamin K-free diet were studied: group (a), cecectomized and fed 1.0% sulfasuxidine (diet 12); group (b), cecectomized and fed no sulfasuxidine (diet 11); group (c), unoperated and fed 1.0% sulfasuxidine (diet 12); group (d) unoperated and fed 1.0% sulfasuxidine plus 75 mg. 2-methyl-1, 4-naphthoquinone per 100 gm. of diet (diet 12K).

Table 3 shows the principal results. A high incidence of hypoprothrombinemia occurred when sulfasuxidine was fed to cecectomized rats while the incidence of hypoprothrombinemia was low when the sulfonamide was not included in the diet of similarly operated animals. The prothrombin time was elevated in fourteen out of the seventeen rats in group (a). Out of nine in group (b) only two had prothrombin times that were higher than normal, and these were only slightly elevated. The average prothrombin time of cecectomized rats fed sulfasuxidine was 68 sec. The average for those cecectomized but not fed sulfasuxidine was only 38 sec., which, according to our procedure, is essentially normal. Moreover, nearly all the rats in the former group eventually died of pericardial hemorrhages following cardiac puncture, but none of the latter

group succumbed or showed any signs of hemorrhage following cardiac puncture.

In addition, animals fed sulfasuxidine and the same basal diet (diet 12), but with intact ceca, group (c) showed scarcely any evidence of vitamin K deficiency (table 3). One of the seven in this group had a slightly elevated prothrombin time during the latter part of the experiment but this soon returned to normal. Another rat in this group, not represented in the table, suddenly became prostrate and was found to be suffering from an internal hemorrhage. The prothrombin time of

TABLE 3

Effect of cecectomy on the prothrombin time of sulfasuxidine-fed rats on a vitamin K-free diet.

DIET NO.	NO. OF RATS	AVG. WEEKS ON DIET	AVG PROTHROMBIN TIME (PT)				TOTAL NO. OF PTS [1] DETNS	NO. OF PTS [1] OVER 50 SEC	NO. OF RATS WITH 1 OR MORE PT OVER 50 SEC.
			4–6 weeks sec.	7–9 weeks sec.	10–12 weeks sec.	Mean sec.			
				Cecectomized					
12	17	10.0	63	68	74	68	72	35	14
11	9	10.3	35	36	43	38	29	2	2
				Not cecectomized.					
12	7	11.5		36	39	38	14	1	1
12K [2]	11	10.5	33	41	35	36	27	1 [3]	1 [3]

[1] Prothrombin time (or times).

[2] 75 mg. 2-methyl-1, 4-naphthoquinone added per 100 gm. diet.

[3] The actual prothrombin time was 51 sec.

this animal was not determined. Its blood did not clot and the hemorrhage was attributed to prothrombin deficiency. Therefore, two animals out of a group of eight had prothrombin deficiency, an outcome almost identical with that of cecectomized rats not fed sulfasuxidine.

A total of eleven control rats with ceca intact received diet 12K (group (d), table 3). Twenty-seven prothrombin time determinations were made. The average was 36 sec. Control animals, not fed the synthetic vitamin K, had an average of 38 sec. This is additional evidence that, with the type of diet

used, intact (unoperated) animals fed sulfasuxidine tend to maintain essentially normal prothrombin levels. However, one animal fed the synthetic vitamin K had one prothrombin time slightly prolonged, namely 51 sec. The two succeeding prothrombin times of this rat were in the normal range. Whether the unexpected high value was actually due to decreased prothrombin content at the time or was caused by some technical error cannot be decided.

The growth and appearance of the cecectomized animals was normal, being similar to that of unoperated controls. Apparently the diets (11 and 12) were adequate except for vitamin K.

TABLE 4

Effect of 2-methyl-1, 4-naphthoquinone (menadione) on the prothrombin time of individual rats.

DIET NO.	TREATMENT	SEX	WKS. ON DIET BEFORE FEEDING MENADIONE	PROTHROMBIN TIME		DAYS AFTER FEEDING MENADIONE
				Before feeding menadione	After feeding menadione	
				sec.	*sec.*	
12	unoperated	♂	7	101	45	7
12	unoperated	♀	7	62	43	7
12	cecectomized	♂	7	60	38	7
12	cecectomized	♂	7	89	38	14
12	cecectomized	♂	12	73	31	2
12	cecectomized	♂	12	45	34	3
12	cecectomized	♂	11	77	32	2
11	cecectomized	♂	10	40	36	2
11	cecectomized	♂	10	37	30	3
9	unoperated	♀	6	70	30	7

Alleviation of hypoprothrombinemia with 2-methyl-1, 4-naphthoquinone

The hypoprothrombinemia, produced under the above described conditions, was undoubtedly due to vitamin K deficiency. In table 4 this is shown by data from ten different rats. After the experiment had continued 6 to 12 weeks synthetic vitamin K was added at a level of 75 mg. per 100 gm. of diet. In animals with elevated prothrombin time normal values were obtained within 2 to 7 days after administration of synthetic vitamin K (table 4).

DISCUSSION

It is significant that so much variability should occur in the incidence of vitamin deficiency when groups of rats are fed sulfasuxidine. This has been noted in all our studies dealing with this sulfonamide. For instance, three of seventeen cecectomized rats given sulfasuxidine, but no vitamin K, remained totally without evidence of hypoprothrombinemia for the duration of the experiment, 10 to 12 weeks, even though the prothrombin time of all the remaining fourteen became greatly elevated. Such variability in response does not occur in animals fed diets deficient in factors which cannot be synthesized in the intestinal tract, i.e., inorganic elements. It indicates that there is a variable degree of vitamin synthesis by intestinal microorganisms as well as variation in coprophagy and other factors promoting the acquirement of certain vitamins. Light et al. ('42) found marked variability in the growth response of rats fed a vitamin B complex-free diet containing 0.5% sulfaguanidine. A somewhat similar effect was reported by Woolley ('42) who observed occasional spontaneous alleviation of inositol deficiency in mice. The intestinal flora of such animals were capable of synthesizing inositol in vitro whereas in those mice continuing to exhibit symptoms of inositol deficiency the flora could not effect such synthesis.

The difference between ether-extracted casein hydrolysate and "vitamin-free" casein in the production of hypoprothrombinemia cannot be satisfactorily explained. It seems probable that the difference might be due to differences in the effect of the two sources of amino acids on the activity of the intestinal microorganisms which synthesize vitamin K. In some unreported experiments rats fed unextracted casein hydrolysate as a source of amino acids seemed to show as high an incidence of hypoprothrombinemia as those given the extracted material. This seems comparable to the findings that raw starch, dextrin or lactose are more conducive to the synthesis of certain B complex vitamins than glucose or sucrose. (Guerrant et al., '35; Tange, '39; Taylor et al., '42).

The data reported here should be considered in connection with the recent studies of Gant et al. ('43) on the concentration of E. coli in the feces and ceca of rats fed purified diets containing sulfasuxidine for different periods of time. In their studies the E. coli count decreased within 3 days after giving sulfasuxidine and remained low for 10 days. Thereafter it began to increase. After 5 weeks it was near the normal level in rats not given a source of "folic acid", but in those which did receive "folic acid" the count remained low. Possibly in our animals given purified diets, which were low in "folic acid", synthesis of vitamin K after the fourth or fifth week was sufficient to account for the failure of hypoprothrombinemia to occur in some. However, Welch and Wright ('43) report that the prothrombin time is decreased by the addition of "folic acid" to the diet of rats fed sulfasuxidine. It should be noted that most of our cecectomized rats receiving sulfasuxidine had high prothrombin time as late as the twelfth week and several eventually died of hemorrhages. These animals were undoubtedly receiving considerable amounts of "folic acid" because the basal diet contained 7% yeast. If some form of resistance to sulfasuxidine tends to develop in the intact rat fed the drug and is manifested by an approach to normal in the activity of bacteria such as E. coli, our data indicate that such does not occur within a period of 12 weeks in cecectomized rats. Hence any tendency toward sulfonamide resistance, possibly through increased synthesis of p-aminobenzoic acid (Landy et al., '43), might be greatly reduced in degree by removal of the cecum or other loci of great bacterial activity.

We did not study the well established inhibiting effect of p-aminobenzoic acid on the bacteriostatic action of sulfaguanidine (Black, McKibbin and Elvehjem, '41), but we did find that the action of sulfasuxidine is antagonized by p-aminobenzoic acid. However, the practically complete absence of hypoprothrombinemia in rats fed ether-extracted yeast and sulfasuxidine cannot be satisfactorily explained on the basis of the p-aminobenzoic acid present in such yeast since it prob-

ably furnished far less p-aminobenzoic acid than was fed in the purified diets (Blanchard, '41). If the yeast exerted its effect by promoting vitamin K synthesis in the alimentary tract the action might have centered in the cecum because cecectomized animals fed the yeast-containing diet and sulfasuxidine had a high incidence of hypoprothrombinemia, whereas the incidence in unoperated control rats was practically nil.

Our results confirm the findings of Griffith ('35) and of Taylor et al. ('42) that rats receiving a good diet do not appear to be handicapped by absence of the cecum. Some of our animals on diet 11 were observed under experimental conditions for about 12 weeks without discernible abnormality. Moreover, certain of these were maintained on a stock diet for over 12 additional weeks without any deviation from normal. Marked hypertrophy and dilatation of the ileum and colon at the site of the anastomosis occurred in the cecectomized animals. This confirms the observation of Griffith ('35) and of Taylor et al. ('42).

That the cecum, or comparable structures favorable to bacterial activity, may be indispensable under some circumstances is clearly shown. The results indicate that, unless the diet is adequate, severe deficiency states may occur when bacterial activity is reduced in the intestinal tract through the bacteriostatic effect of sulfonamides and/or the absence of natural "fermentation vats" such as ceca or rumina.

SUMMARY

Young cecectomized rats fed 1% sulfasuxidine in a vitamin K-free diet containing yeast show a high incidence of severe hypoprothrombinemia. Similarly operated animals fed the basal diet alone, without sulfasuxidine, exhibit a very low incidence of hypoprothrombinemia. Likewise, unoperated rats fed the same basal diet with sulfasuxidine added show only a low incidence of hypothrombinemia. The hypoprothrombinemia is readily alleviated by feeding 2-methyl-1, 4-naphthoquinone. Cecectomized rats appear to maintain good nutritional status if the diet is fully adequate. The data show that

the cecum is an important site of vitamin K synthesis but that this vitamin can be formed in other parts of the intestinal tract.

Young rats fed 1% sulfasuxidine in a purified diet containing B vitamins but no p-aminobenzoic acid develop hypoprothrombinemia and show great restriction in growth. The addition of p-aminobenzoic acid markedly reduces the incidence of hypoprothrombinemia and decreases the restriction on growth. This indicates that p-aminobenzoic acid partially counteracts the effect of sulfasuxidine on vitamin synthesis in the intestinal tract.

Hypoprothrombinemia and impairment of growth may occur to some extent in young rats fed vitamin K-free purified diets containing only 0.08% sulfasuxidine. These effects are more pronounced when the level of sulfasuxidine is raised to 1%.

Hypoprothrombinemia is more prevalent when hydrolyzed casein, instead of "vitamin-free" casein, is the source of amino acids in purified diets containing sulfasuxidine.

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FURTHER STUDIES ON THE COMPARATIVE VALUE OF BUTTER FAT, VEGETABLE OILS, AND OLEOMARGARINES [1]

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Data showing the superiority of butter fat as compared with the vegetable oils for the growth of young rats have been published previously (Schantz, Elvehjem and Hart, '40). This experimental work was carried out with raw skimmed milk as the basal portion of the ration into which were homogenized the fats at a 4% level. Experiments have also been completed with an ether-extracted skim milk powder as the basal portion of the ration (Boutwell, et al., '43). This extraction procedure was followed in order to minimize the amount of butter fat in the vegetable oil rations. Distinct differences in growth favorable to butter fat were obtained. Studies on the effect of various carbohydrates demonstrated that when the lactose was replaced by other carbohydrates, such as sucrose, dextrose, starch, or dextrin, the superiority of butter fat as compared to corn oil disappeared. The ration employed for these comparisons contained 32% of carbohydrate and 28% of fat.

Unpublished work from this laboratory showed that as the level of lactose was increased, the difference in growth rates between rats fed butter fat and those fed corn oil became greater, and therefore, a ration having 48% carbohydrate was used in the present studies. This level of lactose is between the 32–40% found in cow's milk and the 50–55% found in human milk (dry basis). Various vegetable oils and animal fats including butter fat were studied on this ration when the carbohydrate portion was entirely lactose, and when a mixture of carbohydrates was substituted. This mixture was modeled after the mixed carbohydrate diet such as man ordinarily consumes, and the results of this work led to an investigation of oleomargarines on similar rations.

EXPERIMENTAL

The ration used consisted of:

Carbohydrate 48%
Fat 28%
Casein (fat free) 20%
Salts IV[2] 4%

Vitamins added per 100 gm. of ration:

	mg.
Thiamine	0.500
Riboflavin	0.500
Nicotinic acid	0.625
Pyridoxine	0.625
Calcium pantothenate	5.000
p Aminobenzoic acid	30.00
Inositol	100.0
Choline chloride	250.0
Calciferol	.014
β-carotene	.560
α-tocopherol	2.24
2-methyl-1, 4-naphthoquinone	.21

Extraction of the casein with boiling 95% ethanol for two 4-hour periods, followed by two exhaustive diethyl ether extractions, insured against foreign fat contamination. The rations were made up weekly and stored under refrigeration

[2] Phillips, P. H., and E. B. Hart. J. Biol. Chem. vol. 109, p. 657, 1935.

between daily feeding periods. Ad libitum feeding was practiced.

The experiments with the various vegetable oils and animal fats were set up in two parallel series, each consisting of nine groups of six male 21-day-old rats of the Sprague-Dawley strain. One of the series received lactose as the sole carbohydrate, and the other received as the 48% carbohydrate portion a mixture with the following constitution: lactose 3, sucrose 15, dextrose 5, starch 15, and dextrin 10 parts respectively. One of the following fats or oils was fed to each of the nine groups

TABLE 1

Average weights at the end of 5 weeks on both the lactose and mixed carbohydrate rations. The figures represent the average weight in grams of the six male rats in each group.

	BUTTER FAT	CORN OIL	COCONUT OIL	COTTON-SEED OIL	SOY-BEAN OIL	OLIVE OIL	PEANUT OIL	CRISCO	LARD
Lactose ration	169	136	154	155	138	139	138	156	163
Mixed carbohydrate ration	199	193	219	200	204	184	199	200	202

of animals in each series: butter fat obtained by decantation from fresh unsalted sweet-cream butter from the University of Wisconsin Creamery, corn oil[3], coconut oil, cottonseed oil (winter-pressed), soybean oil, peanut oil,[4] olive oil, hydrogenated cottonseed oil[5]. Table 1 shows the results of this experiment expressed as the average weight of the six rats in each group at the end of 5 weeks. While the records shown are for six male rats in each group, we have data for twenty-four animals on both butter fat and corn oil-lactose rations. The average results are not different from the figures on six male rats given in this paper.

[3] Mazola.

[4] Planter's Hi-Hat.

[5] Crisco.

In order to determine whether or not the difference in weight between the butter fat and corn oil groups on the lactose regime was due to the deposition of more fat in the case of the butter fat group, the six rats from each group were killed, the gastro-intestinal tracts removed, and after pooling the carcasses into two respective groups, analyzed for total fat, protein and water content. A similar procedure was followed with the rats fed the butter fat mixed carbohydrate ration to determine whether the kind of carbohydrate fed influenced the relative amounts of these constituents, and therefore afforded a partial explanation for the superior growth on the mixed carbohydrate rations. These data are given in table 2.

TABLE 2

Results of gross analysis of rats. Each figure represents the per cent obtained by pooling the six rats in each group.

RATION	MOISTURE	FAT (DRY BASIS)	PROTEIN (DRY BASIS)
	%	%	%
Butter fat mixed carbohydrate ration	61.6	39.2	51.6
Butter fat lactose ration	65.1	34.8	53.5
Corn oil lactose ration	65.2	32.8	55.0

Using the experimental procedure described above, various commercial oleomargarines were compared to butter fat and corn oil on both the lactose and mixed carbohydrate rations. The oleomargarines were freed of water and non-fatty material by decantation, similar to the preparation of the butter fat. Six male rats 21 days of age were placed on each of the following fats and oils: butter fat, corn oil, three different oleomargarines (oleos A-1, A-2, and A-3) labeled by the manufacturer to be mixtures of animal fats, and four oleomargarines (oleos V-1, V-2, V-3 and V-4) labeled to be mixtures of hydrogenated vegetable oils. One such series of nine groups of animals received lactose as the sole carbohydrate, while in a parallel series of nine groups the carbohydrate mixture already described was fed. Ad libitum feeding was

practiced, and consumption records were kept. Table 3 gives the results of these experiments in terms of the average weight of the six rats in each group at the end of 6 weeks. Table 4 shows the results of the food consumption records expressed in terms of per cent efficiency. These figures were obtained by

TABLE 3

Average weights in grams at the end of 6 weeks of rats fed butter fat, corn oil, and various oleomargarines on both the lactose and mixed carbohydrate rations. Each figure represents the average of six male rats.

	BUTTER FAT	CORN OIL	OLEO A-1	OLEO A-2	OLEO A-3	OLEO V-1	OLEO V-2	OLEO V-3	OLEO V-4
Lactose ration	219	177	212	214	206	212	202	193	194
Mixed carbohy-drate ration	241	224	252	240	224	242	224	246	250

TABLE 4

Per cent efficiency for rats fed butter fat, corn oil, and various oleomargarines on lactose and mixed carbohydrate rations.

$$\text{Per cent efficiency} = \frac{\text{grams gain}}{\text{grams consumed}} \times 100$$

PERIOD	CARBOHY-DRATE	BUTTER FAT	CORN OIL	OLEO A-1	OLEO A-2	OLEO A-3	OLEO V-1	OLEO V-2	OLEO V-3	OLEO V-4
1 to 3 weeks inclusive	Lactose	42	29	43	33	37	35	35	32	33
	Mixture	45	44	46	45	43	44	46	44	47
1 to 6 weeks inclusive	Lactose	39	36	41	34	37	37	35	32	34
	Mixture	41	41	42	40	39	40	39	42	41

dividing the number of grams gained during a given period by the number of grams of food consumed during that period and multiplying by 100. The periods chosen for this report are the first to third weeks inclusive and the first to sixth weeks inclusive.

DISCUSSION

The data given in table 1 clearly show that from a nutritional standpoint, an interrelationship between the kind of carbohydrate and the kind of fat exists, and this fact must be considered in obtaining the correct nutritional value of either

nutrient. With lactose as the sole carbohydrate, the vegetable
oils were definitely inferior to butter fat and lard. Thus the
rats receiving butter fat on the lactose diet grew 33 gm. more
during a period of 5 weeks than those fed corn oil. The other
vegetable oils tested ranged from 15 to 32 gm. below butter
fat, while lard, which is an animal fat, gave a figure of only
6 gm. below butter fat. These results substantiate our earlier
observations (Boutwell, et al., '43) that with lactose as the
only carbohydrate, animal fats including butter fat were su-
perior to vegetable oils in the nutrition of young rats. That
the kind of carbohydrate is important can also be seen from
table 1. With the mixture of carbohydrates substituted for
lactose, approximately equal growth was obtained by the rats
fed either animal fats or vegetable oils, and furthermore,
growth for each group was much greater on the mixture than
on lactose. No apparent reason can be given for the superior
growth of the rats fed coconut oil nor the inferior growth
of the rats fed olive oil when the carbohydrate mixture was
used. Table 3 shows that the only marked difference in gross
analysis between the rats fed lactose and those fed the mix-
ture of carbohydrates was in the total per cent of fat. On the
carbohydrate mixture the butter fat animals averaged 39.2%
of fat, while on the lactose regime the butter fat and corn
oil groups averaged 34.8 and 32.8% respectively. This can
be interpreted as meaning that on the carbohydrate mix-
ture more carbohydrate is available for fat formation than
on the lactose diets, and that perhaps more fat is formed
and deposited than when lactose is the sole carbohydrate in
the ration. Such an interpretation could account in part for
the superior growth on the carbohydrate mixture in compar-
ison to that on lactose. The differences in gross analysis
between the butter fat and corn oil groups on the lactose
ration are not sufficiently large to account for the superior
growth of butter fat fed rats in comparison to those fed corn
oil, and by analogy, in comparison to the animals fed any of
the vegetable oils tested.

These data are related directly to the question of the desirability of allowing the manufacture of a "filled milk" which may become the sole source of nutrition for the growing child. The data make it apparent that a great responsibility would be thrown on those who believe that such a product as "filled milk" as the sole article of nutrition could function adequately in early nutrition.

Unpublished data have shown definitely that "filled milks" are inferior to whole milk in growth promoting properties when fed to young rats. By a "filled milk" we mean a skimmed milk fortified with a vegetable oil to the same fat level as a whole milk.

It is evident from the above data that the results obtained on the mixed carbohydrate diet would conform more closely to the carbohydrate intake of the ordinary human diet, than would the results on the lactose diet. In the mixed diet of man starches, sugars and a variety of other carbohydrates are consumed. Potatoes, breads, cane sugar, and molasses all contribute starch, sucrose, or dextrose to the diet. Consequently, it becomes possible to throw some light on the much discussed question as to the comparative nutritive value of oleomargarines of either vegetable or animal origin when reinforced with vitamin A and fed in a ration complete in all other nutrients.

Table 4 shows that with lactose as the sole carbohydrate the animal oleomargarines were but slightly below butter fat in nutritive value, while all but one of the vegetable oleomargarines were distinctly inferior. The poorest growth was obtained with the corn oil ration. These results are in keeping with the thesis that the animal fats investigated are superior to the vegetable oils for the early growth of rats on a lactose ration. On the mixed carbohydrate diet, such as man ordinarily consumes, there was no conclusive evidence that oleomargarines were inferior to butter fat. In fact, some oleomargarines showed slight superiority and there was quite as much variation among oleomargarines as between the butter fat and the oleomargarines. If 6 weeks of growth

of the rat at a rapid stage of its development can be used as the criterion of adequate nutrition, then certainly these oleomargarines compare favorably with butter fat. Whether this deduction is entirely sound can be answered only when growth to maturity and reproduction are studied experimentally.

The results given in table 4 show that on the lactose diet the rats receiving butter fat, oleo A-1 or oleo A-3 were more efficient in their utilization of food during the first to third week period in comparison to those receiving corn oil or one of the other oleomargarines tested. Thus all but one of the fats of animal origin were superior on an efficiency basis to those of vegetable origin during this early period. At the end of 6 weeks the picture remained relatively the same, though the corn oil and oleo V-1 groups had improved somewhat. With the carbohydrate mixture in the diet, however, there were no marked differences in efficiencies between the various groups and in all cases the efficiency values were higher than the corresponding group receiving only lactose as the carbohydrate. The over-all picture in respect to efficiency data is, therefore, similar to that shown by the growth records. The higher efficiency of the rats on the mixed carbohydrate diet plus the fact that more fat was deposited than in the case of the lactose rations (table 2) strongly indicate that the mixed carbohydrate is more readily utilized, and if such is the case, more fat is synthesized, with the end result that the importance of the dietary fat is lessened and all groups grew the same. A more detailed investigation is necessary before a definite conclusion can be reached on this phase of the problem.

SUMMARY

1. With lactose as the sole carbohydrate in the diet, rats showed superior growth when fed butter fat or lard as compared to corn oil, coconut oil, cottonseed oil, soybean oil, peanut oil, olive oil and hydrogenated cottonseed oil .

2. With a mixture of carbohydrates composed of sucrose, starch, dextrose, dextrin, and lactose in the diet, the average

growth response of the animals fed vegetable oils was equal to that of the animals fed butter fat and lard. The growth rate on this ration was more rapid than when all of the carbohydrate was present as lactose.

3. Properly fortified oleomargarine fats gave growth equal to butter fat over a period of 6 weeks when the above mixture of carbohydrates was incorporated in the rations.

4. Properly fortified oleomargarines did not give growth equal to butter fat when lactose was the sole carbohydrate in the diet. On such a regime rats fed butter fat grew slightly better than rats fed oleomargarines of animal origin, but decidedly better than rats fed oleomargarines of vegetable origin.

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THE EFFECT OF HIGH TEMPERATURE AND B-VITAMIN LEVELS OF THE DIET UPON THE METABOLISM AND BODY COMPOSITION OF RATS [1]

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Mills ('41 and '42) has reported that for optimal growth of rats maintained at 91°F. and fed ad libitum, the concentrations of thiamine, choline and pyridoxine needed in the diet were greater than those required at 65°F. The optimal levels of riboflavin and pantothenic acid were the same at both temperatures.

In the present work, the effect of a high environmental temperature upon the metabolism and utilization of some of the B vitamins in the rat were studied in an attempt to gain more information concerning the requirements for and utilization of these B vitamins at high temperatures. Growth, urinary excretion of nitrogen and some B vitamins and body composition were determined in four groups of rats maintained at 75° and 91°F. on diets containing two levels of B vitamins, one slightly above minimal requirements, the other greatly in excess. The food intake of the groups at lower temperature was restricted to that of the corresponding groups at 91°F., which were fed ad libitum.

EXPERIMENTAL

Twenty male rats (age 30 days) weighing 60-91 gm. were selected for four groups of five, each group similar in distribution of weights and averaging 71 gm. per rat. The groups were placed in metabolism cages and fed purified diets low and high in the vitamin B-complex. The diets were made up as follows: purified casein[3], 180 gm.; corn starch, 220 gm.; sucrose, 420 gm.; beef suet, 80 gm.; cod liver oil, 40 gm.; B. D. H. mixture[4], 40 gm.; ruffex[5], 20 gm.; choline hydrochloride, 2.5 gm.; and concentrated liver extract[6] in such amounts as to provide for the "low vitamin" and "high vitamin" diets, respectively, 2 and 4 mg. of thiamine, 2 and 4 mg. of riboflavin, 4 and 8 mg. of pantothenic acid, 6.6 and 13.2 mg. of nicotinic acid, and 1.4 and 2.8 mg. of pyridoxine. Water was added to give 5 kg. of cooked diet. The diets were cooked as previously described (Sarett, Klein and Perlzweig, '42; Sarett and Perlzweig, '43) and contained 2 levels of the vitamin B-complex supplied as a supplemented liver concentrate. The choline content was the same for both diets. Groups A and B received the diet high in B-complex and groups C and D the lower vitamin diet.

All four groups were kept at room temperature (75°F.) for 5 days during which body weights and urine analyses were checked to determine the extent of agreement of the results yielded by similar groups. At this time the average weight of the rats in groups A, B, C and D were 104, 106, 99 and 101 gm. respectively. The cages containing groups A and C were then transferred to a large insulated dog cage which was maintained at 90–91°F. by means of covered electric bulbs, two of which were attached to a thermostatic control. No attempt was made to regulate or increase the relative humidity which

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[3] Labco.

[4] Prepared according to Drummond and Watson ('22).

[5] Fisher Scientific Company.

[6] Generously supplied by Lederle Laboratories, Pearl River, New York. The extract of each 10 gm. of fresh liver was supplemented with synthetic vitamins to contain the following: thiamine, 1 mg.; riboflavin, 1 mg.; calcium pantothenate, 2 mg.; nicotinic acid, 3.3 mg.; and pyridoxine, 0.7 mg.

was usually about 20%. Fresh water and 2% NaCl solution were available to all four groups of animals. Saline intake is reported in table 1.

The food intakes of the animals at room temperature (B and D) were restricted to the previous day's consumption by the corresponding group at high temperature (A and C). The animals at room temperature were therefore eating less than they would have eaten normally. The high vitamin group at high temperature (A) ate about 25% more food than the low vitamin group at high temperature (C). Since the B

TABLE 1

Food intake and weight gain per rat per day during experimental period at 75° and 91°F.

GROUP	WEIGHT GAIN	INTAKE							
		Calories	Protein	Thiamine	Riboflavin	Pantothenic acid	Nicotinic acid	Choline	2% NaCl
	gm.	*cal.*	*gm.*	*µg.*	*µg*	*µg*	*µg*	*mg*	*ml.*
A-High Vitamin, 91°F.	3.6	37.4	1.53	37.7	37.7	75.4	124	21	13
B-High Vitamin, 75°F.	3.1	37.5	1.53	37.9	37.9	75.8	125	21	13
C-Low Vitamin, 91°F.	2.2	28.4	1.15	14.5	14.5	29.0	48	16	18
D-Low Vitamin, 75°F.	1.6	28.6	1.17	14.6	14.6	29.2	48	16	24

vitamin concentration of the high diet was twice that of the low diet, the actual vitamin intake of groups A and B was about two and one-half times the vitamin intake of groups C and D. The average daily food intake and weight gain during the experimental period are given in table 1. On the twenty-second day, groups A and B were fasted for 24 hours and then sacrificed for analysis. Groups C and D were analyzed similarly after the twenty-fifth day.

Urines were collected daily and analyzed for nitrogen, riboflavin, panothenic acid, nicotinic acid and trigonelline. Nitrogen was determined by Kjeldahl method, nicotinic acid and trigonelline by the method of Perlzweig, Levy and Sarett ('40) and pantothenic acid and riboflavin by microbiological methods (Pennington, Snell and Williams, '40, Snell and Strong, '39). In the pantothenic acid assay the yeast supple-

ment of Strong, Feeney and Earle ('41) was substituted for the alkali treated yeast extract.

Sacrifice of the animals and preparation of liver and carcass for analysis were carried out as previously described (Sarett and Perlzweig, '43). Nicotinic acid was determined chemically (Dann and Handler, '41), nitrogen by Kjeldahl, and fat by alkaline digestion, acidification, extraction with ether and weighing. Riboflavin and pantothenic acid were determined microbiologically as in the urine, after digestion with papain and takadiastase at pH 4.5 and filtration through supercel (Cheldelin et al., '42). The filtrates were extracted with ether to remove interfering fats (Bauernfeind, Sotier and Boruff, '42) before the pH was adjusted for the assays. For thiamine the thiochrome method of Mason and Williams ('42) was directly applied to a portion of these enzymatic digests without the use of permutit. A slight excess of ferricyanide was used to insure complete oxidation to thiochrome and checked by recoveries of added thiamine. Blanks were obtained by sulfite treatment.

Effect of high temperature upono the urinary excretion of nitrogen and B-vitamins

Table 2 shows the average daily excretion per rat of nitrogen, riboflavin, pantothenic acid, nicotinic acid and trigonelline by each of the four groups during the experimental period.

TABLE 2

Urinary excretion per rat per day of nitrogen and B-vitamins during experimental period at 75° and 91°F.

GROUP	NITROGEN	RIBO-FLAVIN	PANTO-THENIC ACID	NICOTINIC ACID AND TRIGONELLINE
	mg	*μg.*	*μg.*	*μg.*
A–High Vitamin, 91°F.	102	12.9	7.1	281
B–High Vitamin, 75°F.	116	12.2	4.8	277
C–Low Vitamin 91°F.	72	2.1	3.1	188
D–Low Vitamin, 75°F.	87	1.7	2.4	181

A comparison with table 1, which shows the daily intake of these substances, gives an estimate of the amounts retained by the rat. The feces were not analyzed. The nitrogen excretion of the rats at room temperature was higher than that of the corresponding group at 91°F. This was to be expected since the caloric requirements were higher at the lower temperature and is corroborated by the slower growth and lower nitrogen and fat content of the rats at room temperature. However, the rats at high temperatures at both levels of vitamin intake excreted about the same amounts of riboflavin and more pantothenic acid than the corresponding group at room temperature. During the first week the rats at high temperature excreted more riboflavin and pantothenic acid than their respective control groups. For the remainder of the experimental period only the pantothenic acid excretion of the groups on the higher vitamin intake differed markedly; small but insignificant differences were obtained for excreted pantothenic acid at low vitamin intake and for riboflavin at both levels of intake. All four groups excreted more nicotinic acid and trigonelline than their nicotinic acid intake. This extra excretion was about the same for all groups regardless of temperature or B-vitamin content of the diet.

The effect of high temperature upon the body composition of the rat

Table 3 gives the average composition of the livers and carcasses of each group of five rats after the experimental period at 75° and 91°F. Groups A and B were sacrificed after 22 days, C and C after 25 days. The rats kept at high temperature gained more weight than their corresponding control group. The livers of the control groups, however, especially on the high vitamin diet, constituted a greater percentage of the total body weight.

The concentrations of water in the carcasses of the animals kept at 91°F. were significantly lower than in those at 75°F.

TABLE 3

Average composition of rat liver and carcass after experimental period at 75° and 91° F.

GROUP	TISSUE ANALYZED	WEIGHT	WATER	NITROGEN PER GRAM	FAT	ASH	RIBO-FLAVIN PER GRAM	PANTO-THENIC ACID PER GRAM	THIAMINE PER GRAM	NICO-TINIC ACID PER GRAM
		gm.	*%*	*mg.*	*%*	*%*	*µg.*	*µg.*	*µg.*	*µg.*
A 22nd day High Vitamin, 91°F. (5 rats, 183 gm. average weight)	Carcass	161	62.7	25.5	14.7	3.0	3.5	9.7	1.67	42.8
	Liver	7.8	74.0	28.8	4.2		23.2	75.7	6.0	132
B High Vitamin, 75°F. (5 rats, 174 gm., average weight)	Carcass	148	65.4	26.5	12.3	3.3	2.6	7.4	1.35	44.5
	Liver	9.2	73.2	28.1	4.1		22.	68.9	5.1	124
C 25th day Low Vitamin, 91°F. (5 rats, 155 gm., average weight)	Carcass	134	65.9	26.7	12.3	3.4	2.1	5.4	.82	40.0
	Liver	6.1	73.6	29.5	4.6		20.	61.	3.9	130
D Low Vitamin, 75°F. (5 rats, 140 gm., average weight)	Carcass	123	68.4	27.0	8.5	3.6	2.3	6.9	.97	42.5
	Liver	6.1	73.7	31.1	3.9		21.8	59.4	4.3	129

and were compensated by the increased fat content. The lower fat content at room temperature was presumably due to need for more calories. While the animals of the two groups kept at 91° retained more nitrogen, the concentration of nitrogen in the carcasses of these animals was the same or slightly lower than in their respective control groups. The concentrations of water, fat and nitrogen in the livers of these animals showed no significant differences.

The concentrations of riboflavin, pantothenic acid and thiamine in the carcasses were highest in the group at high temperature at the high level of B-vitamin intake and lowest in the other high temperature group whose diet contained one-half of this B-vitamin level. These differences from the groups at room temperature were significant when analyzed by the t test (Snedecor, '38). Both groups at room temperature had the same concentration of riboflavin and pantothenic acid in their tissues, while the thiamine content varied with the intake. This agrees with the previous observations of Sarett and Perlzweig ('43). The concentrations of riboflavin, pantothenic acid and thiamine in the livers of these animals followed the same pattern as in the carcasses, the only significant differences being that the highest levels of pantothenic acid and thiamine were in group A. The nicotinic acid concentrations were essentially the same in the livers and in the carcasses of all four groups.

DISCUSSION

At levels of intake above the minimum requirements for the rats at room temperature the riboflavin, pantothenic acid and thiamine concentrations in the carcasses of rats kept at high temperature varied with the concentrations of these vitamins in the diet. At room temperature, rats on these diets restricted to the same caloric intake as the respective groups at 91°F. showed no variation in the riboflavin and pantothenic acid concentrations in the carcasses and only the tissue thiamine varied with the level of B-vitamins in the diet. Comparing only groups A and C, which were maintained at

91°F., the data show that the increased B-vitamin content of the diet has increased the appetite and growth rate of the rats, which is curtailed by high temperature (Mills, '41 and '42), and resulted in carcass tissues almost twice as rich in riboflavin, pantothenic acid and thiamine. Given the high vitamin diet, the rats at high temperature laid down tissue containing more riboflavin, pantothenic acid and thiamine per gram than normal rats with this excess B-vitamin intake, while with an intake about adequate at room temperature, the tissues laid down at high temperature are very low in these vitamins. These data on tissue composition may lead to the inference that a level of B-vitamin intake considered adequate at moderate environmental temperatures may result in a partial depletion of such vitamins in the tissues at high temperatures.

The data still cannot be interpreted in terms of requirements of these vitamins at high temperatures, since the divergence in tissue content on the two diets is not correlated with the urinary excretion of these vitamins. On both low and high vitamin diets the rats at high temperature excreted as much or more riboflavin and pantothenic acid than did the respective controls at room temperature. Holt ('43) has also found more thiamine in the urine of human subjects during hot weather. The urinary excretion and total body content of riboflavin and pantothenic acid show that the high vitamin group at high temperature has utilized or destroyed less riboflavin and pantothenic acid than its control group, while the low vitamin group at high temperature has utilized or destroyed more of these substances than its control group. At 91°F. the rats required fewer calories than at 75°F. as evidenced by the higher fat content of the tissues and increased nitrogen retention by the animals. The concomitant need for less vitamins for this catabolism and the finding of increased urinary excretion of these vitamins at high temperature would indicate a lower B-vitamin requirement at higher temperatures, but the opposite conclusions might be

drawn from the tissue concentrations of the vitamins in the rats at high temperature on the low vitamin diet.

The similarity in data for riboflavin, pantothenic acid and thiamine in these experiments may indicate that any changes in requirements, that may be demanded by a change in temperature would be similar for all of these vitamins. Mills ('41, '42), interprets his data on the levels of these vitamins needed for optimal growth of rats at high and low temperatures on a synthetic diet fed ad libitum as indicating no differences in riboflavin and pantothenic acid requirement and a doubling of the thiamine requirements at high temperature.

These findings can only be applied to resting animals at higher temperatures and reveal nothing of the vitamin requirements for work or exercise at high temperature. This obviously important question also requires further investigation.

SUMMARY

Groups of rats were maintained for 22–25 days at 91°F. on high and low intakes of the B-vitamin-complex. The corresponding control groups were kept at 75°F. and their food intake was restricted to that of the animals at 91°. The analyses of the urine and of the bodies at the end of the experiment showed that: At both levels of vitamin intake the rats at 91° gained more weight, retained more nitrogen, fat and water than the corresponding controls. The concentration of water was lower and that of fat was higher in the animals at 91°. The concentration of riboflavin, pantothenic acid and thiamine was highest in the bodies of the group on the high vitamin intake kept at 91° and was lowest in the low vitamin group kept at 91°. In the two groups at 75°, the level of intake had no effect upon the concentration of riboflavin and pantothenic acid in the tissues, while the thiamine concentration varied with the intake. The nicotinic acid of the tissues remained uninfluenced either by level of intake or temperature.

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THE RETENTION OF VITAMINS IN VEAL AND LAMB DURING COOKING [1]

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Considerable interest has developed concerning the vita-men retention in meat after various cooking processes. The vitamin retention in pork during storage, curing and cooking has been reported recently (McIntire et al., '43), (Schweigert et al., '43) and (Schweigert et al., '43a). In this paper we wish to report the thiamine, riboflavin and nicotinic acid retention in veal and lamb after different methods of cooking, namely, roasting, braising, broiling and stewing.

EXPERIMENTAL

The samples of veal used in these studies were obtained from veal carcasses weighing approximately 90 pounds. The lamb samples were obtained from lambs weighing 50 pounds, dressed. The veal samples were prepared as follows: The loin was removed by a cut perpendicular to the aitch bone. One sirloin steak was discarded and two adjacent ¾-inch steaks were retained for cooking tests. The shank was removed from the leg at the stifle joint and the rump was removed from the round. The remaining portion of the leg was used for testing. The third and fourth rib steaks were removed from the shoulder for testing, and a boned rolled cut was prepared

from the remainder of the shoulder. In all'cases paired cuts were obtained from both sides of the animal for fresh and cooked samples.

The stewing meat was obtained by cubing muscular sections of the flanks and shanks. The cubes were than thoroughly mixed and equal portions were used for fresh and cooked samples. Corresponding sirloin, leg, and stewing samples were taken from the lamb carcasses.

The cooking was carried out under standard procedures without seasoning as described in "Meat and Meat Cookery," ('42). The braising method has been described by McIntire et al. ('43). The veal rib and sirloin steaks were braised for 1 hour. Approximately 30 minutes were required to broil lamb steaks. The steaks were placed on a rack 10 inches from the heating unit and when the internal temperature reached 55°C., the steaks were turned and allowed to reach a final internal temperature of 77°C. Roasting time for veal shoulder roasts ranged from 2 hours and 22 minutes to 4 hours and 17 minutes, and for veal leg roast from 3 hours and 45 minutes to 5 hours. The roasting time for leg of lamb was 2 hours and 56 minutes and 4 hours and 32 minutes for the two roasts. Stewing was carried out in a covered iron kettle. The water was heated to boiling and poured on the meat in proportions of 1:2 by weight. The temperature of the stew was allowed to reach 85°C. and maintained for 2 hours.

In every case the drippings and stewing water were retained for analysis. Preparation and storage of the meat samples and drippings and analyses for moisture and fat were carried out as reported by McIntire et al. ('43).

The thiamine content of the samples was determined by the method of Hennessy ('42) with modifications by McIntire et al. ('43). The nicotinic acid was determined by the method of Snell and Wright ('41), after extraction with 4% alkali. The riboflavin determinations were made by the method of Snell and Strong ('39), with some modifications in the digestion and extraction procedure. A suitable sample of meat was autoclaved for 15 minutes in 50 ml. 0.1 N H_2SO_4. Two-

TABLE 1

Detailed analysis of meats. Three series of veal and two series of lamb were analyzed. Illustrative results of the second veal series and the first lamb series.

	LEG OF VEAL FOR ROASTING	VEAL SIRLOINS STEAKS FOR BRAISING	ROLLED VEAL SHOULDER FOR ROASTING	VEAL RIB STEAKS FOR BRAISING	VEAL FOR STEWING	LEG OF LAMB FOR ROASTING	LAMB SIRLOIN STEAKS FOR BROILING	LAMB FOR STEWING
Before cooking								
Wt. of meat — gm.	2640	465	2029	497	548	1241	376	612
H₂O content — gm.	1957	321	1485	368	408	827	231	386
Fat content — gm.	95	53	120	20.0	14	151	75	103
Res. solids — gm.	589	91	424	108	126	250	70	122
Thiamine content — mg.	4.42	0.84	3.45	0.85	0.78	2.48	0.68	0.83
Riboflavin content — mg.	9.1	1.65	7.42	1.93	2.03	3.72	1.06	1.61
Nicotinic acid content — mg.	222	39	142	37.3	41.2	84.2	21.8	34.9
After cooking								
Wt. of meat — gm.	1853	295	1310	297	348	871	276	399
H₂O content — gm.	1133	141	767	175	216	493	142	210
Fat content — gm.	107	57	127	19.6	17.4	131	64	74
Res. solids — gm.	575	87	415	102	114	246	71	106
Thiamine content of meat — mg.	2.34	0.33	1.76	0.29	0.21	1.38	0.49	0.23
Thiamine content of drippings — mg.	0.24	0.16	0.44	0.14	0.19	0.13	0.69	0.20
Riboflavin content of meat — mg.	7.1	1.24	6.2	1.35	1.3	3.26	0.83	1.16
Riboflavin content of drippings — mg.	0.74	0.38	0.89	0.41	0.6	0.36	0.06	0.53
Nicotinic acid content of meat — mg.	171	23.9	109	22	23	58.4	16.8	18.4
Nicotinic acid content of drippings — mg.	35.6	13.0	26.4	9.5	19.0	15.1	2.2	16.2

TABLE 2

Vitamin retention after cooking.

All values in %.

Determination	FRESH WEIGHT RETAINED			THIAMINE RETAINED IN MEAT			TOTAL THIAMINE RETAINED			RIBOFLAVIN RETAINED IN MEAT			TOTAL RIBOFLAVIN RETAINED			NICOTINIC ACID RETAINED IN MEAT			TOTAL NICOTINIC ACID RETAINED		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Roast leg of veal	75	69	70	70	53	52	77	59	58	90	78	81	97	86	90	86	77	73	100	93	87
Braised sirloin of veal	63	61	61	45	40	35	68	59	..	69	75	74	89	98	..	60	61	57	95	94	..
Roast shoulder of veal	71	64	68	65	51	53	76	63	66	91	83	84	99	95	96	93	77	78	99	96	97
Braised rib of veal	66	60	62	49	36	35	66	51	51	77	70	72	94	91	94	69	60	60	95	85	90
Stew of veal	63	63	62	22	26	28	47	51	54	69	64	65	103	95	106	51	56	47	101	102	95
Roast leg of lamb	71	70	..	53	56	..	58	61	.	82	88	..	94	97	..	77	69	..	97	87	..
Broiled sirloin of lamb	65	73	..	68	72	..	77	82	..	80	78	..	90	84	..	83	77	..	100	87	..
Stew of lamb	64	65	..	26	28	..	50	52	..	68	72	..	100	105	..	52	53	..	105	99	..

tenths gram of papain dissolved in 5 ml. of 2.5 molar sodium acetate was then added to the digest and the mixture was incubated over night. The digest was then neutralized, filtered, and extracted by shaking with ether, (Strong et al., '42), then the aqueous portion was made to a definite volume. The papain digest was used because of its low riboflavin content as compared to clarase.

TABLE 3

Table of analysis of fresh and dry veal and lamb samples.

All values µg. per gram.

| | THIAMINE | | RIBOFLAVIN | | NICOTINIC ACID | |
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Veal — average of three series of samples.						
Fresh leg	1.8	6.8	3.1	11.7	75	283
Roast leg	1.5	3.9	3.9	10.5	83	222
Fresh sirloin	1.9	6.2	3.1	10.1	71	237
Braised sirloin	1.2	2.4	3.7	7.3	69	138
Fresh shoulder	1.7	6.3	3.2	11.4	61	220
Roast shoulder	1.4	3.5	4.0	9.9	71	174
Fresh rib	1.7	6.5	3.3	12.4	62	233
Braised rib	1.1	2.5	3.9	9.3	65	155
Fresh stew	1.6	6.1	3.1	11.7	68	255
Cooked stew	.66	1.7	3.2	8.5	57	150
Lamb — average of two series of samples.						
Fresh leg	2.0	6.50	2.95	9.6	63	204
Roast leg	1.55	3.60	3.54	8.2	65	151
Fresh sirloin	1.72	4.78	2.80	7.0	53	138
Roast sirloin	1.74	3.52	3.20	6.5	61	123
Fresh stew	1.39	3.48	2.70	7.8	58	166
Cooked stew	.59	1.28	2.93	6.4	47	203

Cooking tests have been carried out on three groups of veal samples and two groups of lamb samples. All analyses were made directly on the fresh cooked and uncooked samples. The vitamin content of the dry samples was calculated on the basis of the moisture analysis. Detailed analyses of veal and lamb samples are shown in table 1. The average per cent retention of weight and vitamins for each method of cooking is shown in table 2. The average vitamin contents of the samples from the three veal series and two lamb series are shown in table 3.

All calculations were made according to the method described by McIntire et al. ('43), and Schweigert et al. ('43).

The proximate analyses indicate that almost the entire decrease in weight in the meat during cooking was due to loss of water. Meats roasted and broiled consistently retained more moisture than those braised and stewed. The average range of weight retention for roasting and broiling was from 68 to 71%, while it was 61 to 65% for braising and stewing.

Results for vitamin retention corresponded with water loss in every case. Roasting and broiling procedures resulted in higher retention of the vitamins in the meat than did braising and stewing. The average retention of thiamine after roasting of lamb and veal was 57%; after broiling of lamb, 70%; after stewing of veal and lamb, 26%; and after braising of veal, 40%. The average retention of riboflavin after roasting of veal and lamb was 79 to 86%; after broiling of lamb, 79%; after braising of veal, 73%; and after stewing, 66%. The nicotinic acid retention in the meat was generally lower than riboflavin but higher than thiamine. The average retention of nicotinic acid after roasting was 73% for lamb and 81% for veal; after broiling lamb, 80%; after braising veal, 61%; and after stewing, 52%. These results indicated that nicotinic acid was more loosely bound and more readily extracted than riboflavin.

Average total retention of the vitamins is similar after roasting and braising. The total retention of thiamine after roasting of lamb was 60%, after roasting of veal was 67%; after braising the total retention was 60%. Highest total retention of thiamine resulted after broiling. Eighty per cent of the thiamine was retained after broiling. This supports the results obtained after frying (pan-broiling) of pork by Schweigert et al. ('43). Stewing on the other hand resulted in the lowest total retention of thiamine. Only 51% of the thiamine was retained. This indicates the ability of thiamine thiamine was retained. This indicates the lability of thiamine to heating in aqueous solutions. Total retention of the more

stable vitamins was nearly the same in all cases. The average total retention for riboflavin after the various cooking methods ranged from 87 to 101% and for nicotinic acid from 92 to 100%.

The high total retention of the riboflavin and nicotinic acid indicates that practically all of the loss in the meat alone was due to leaching and extraction. This is borne out by the previously mentioned fact that the vitamin retention closely parallels the moisture retention during cooking. The extent of the vitamin retention can also be correlated with the size of the cuts of meat, the type of cooking and the time required for cooking.

About the same amount of vitamins was retained in the meat after roasting and broiling. The broiled meat was cooked only 30 minutes as compared to 2 to 5 hours required for roasting. However, the steaks are considerably smaller and have much more surface area exposed per unit of weight. The thiamine content of the drippings for the size of the samples was lowest after roasting. This may be attributed to the destruction during the long cooking period. The braising process involves a continual extraction of the meat by the condensing vapors in the kettle. The stewing process is simply a hot water extraction. This likely accounts for the lower vitamin retention in the meats after braising and stewing. Stewing gave the most consistent results of any of the methods studied. This is attributed to more uniform sampling and a more standardized cooking procedure.

The results of the retention studies on veal and lamb are in good agreement with those previously reported on pork (McIntire et al., '43) and (Schweigert et al., '43).

The values obtained for thiamine, nicotinic acid and riboflavin contents of veal and lamb are generally in good agreement with those reported by previous workers. Thiamine values ranged from 1.28 to 2.32 µg. per gram for veal and from 1.35 to 2.0 µg. per gram of fresh lamb. These figures are lower than 3.5 µg. per gram of fresh veal and 3.0 µg. per gram of lamb reported by Waisman and Elvehjem ('41).

Their figures, however, are based on the analysis of meat trimmed of fat and connective tissue and cannot be compared directly with those reported in this paper. The riboflavin values ranged from 2.88 to 3.9 µg. per gram of fresh veal and 2.6 to 3.0 µg. per gram of fresh lamb. These figures are somewhat higher than 2.2 µg. per gram for veal and 2.4 µg. per gram of lamb reported by Cheldelin and Williams ('42). The nicotinic acid values ranged from 49 to 84 µg. per gram of fresh veal and 48 to 68 µg. per gram of fresh lamb. These values compare favorably with 73 µg. per gram of veal and 77 µg. per gram of lamb reported by Dann and Handler ('42), and 68 to 94 µg. per gram of veal and 85 µg. per gram of lamb reported by McIntire et al., ('41).

There are interesting variations in the vitamin content of samples taken from the same carcass. This may be illustrated by values from veal samples of a single carcass. The thiamine content of the veal stewing meat, 1.43 µg. per gram, is lower than the other cuts, 1.67 µg. per gram for leg of veal and 1.7 µg. per gram of veal shoulder. The riboflavin content of the leg and sirloin of veal, 3.45 and 3.55 µg. per gram, respectively, is lower than the riboflavin values for veal shoulder and veal rib, 3.65 and 3.9 µg. per gram respectively. The opposite is true for nicotinic acid. The vitamin content of leg and sirloin cuts, both 84 µg. per gram is higher than for the shoulder cuts, 70 µg. per gram. A leg of lamb contained higher amounts of all three of the vitamins than the lamb sirloin from the same carcass. This was probably due to the difference in the fat content of the two cuts. The per cent of fat in the sirloin, 24%, was more than twice that in leg of lamb, 10%.

SUMMARY

1. Vitamin retention in veal and lamb after cooking has been studied.

2. The retention in the meat alone after roasting and broiling averaged 57 and 70% for thiamine, 82% for riboflavin and 80% for nicotinic acid.

The retention after braising of veal averaged 40% for thiamine, 73% for riboflavin and 61% for nicotinic acid. The retention after stewing averaged 26% for thiamine, 66% for riboflavin and 52% for nicotinic acid.

3. The average total thiamine retention in the meat plus drippings was 62% after roasting and braising, 80% after broiling, and only 51% after stewing. The average total retention after the various cooking methods ranged from 87 to 101% for riboflavin and 92 to 100% for nicotinic acid.

4. The vitamin content of different cuts of meats from the same carcass showed interesting variations. The average thiamine value was 1.7 µg. per gram of fresh lamb and 1.74 µg. per gram of veal. The average riboflavin value was 2.8 µg. per gram for lamb and 3.15 µg. per gram for veal, and the average nicotinic acid value for lamb was 58 µg. per gram and for veal was 67 µg. per gram.

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THE UTILIZATION OF THE SULFUR AMINO ACIDS BY THE CHICK

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Klose and Almquist ('41) have reported the indispensability of methionine in the diet of the chick and have shown that homocystine can replace methionine in the presence of adequate amounts of choline. In these respects the chick is similar to the rat, in which the effect of choline on homocystine utilization as a substitute for methionine was originally reported (du Vigneaud, et al., '39). Binkley and du Vigneaud ('42) have demonstrated the conversion of homocysteine to cysteine by rat liver tissue, and have shown that methionine is less effective than homocysteine in cysteine formation. This evidence has strengthened the theory of demethylation of methionine with subsequent utilization of the homocysteine residue for cysteine formation.

In the present work with chicks, a study has been made of homocystine as a substitute for cystine in the absence of dietary choline, as well as the interrelationships between cystine, homocystine and methionine in the presence of choline. S-Methylcysteine has been investigated as a choline-replacing agent for homocystine utilization and as a cystine-replacing compound. Natural and synthetic methionine have been compared for their ability to support growth.

EXPERIMENTAL

Previous studies (Klose and Almquist, '41; Almquist and Jukes, '42) of the sulfur-bearing amino acids in the diet of the chick have utilized an arachin basal diet which was deficient in methionine and several other indispensable amino acids but carried fairly large amounts of cystine. Studies on raw soybean protein as the only protein in the diet (Almquist et al., '42) have shown that the unavailability of methionine accounts almost entirely for the poor growth obtained with this protein. For this reason, an isolated soybean protein preparation was chosen for the present studies. This material was a commercial product [1] containing 86.6% protein (N × 6.25) and 1.75% ash. The protein contained 1.53 ± .08% methionine and less than .25% cystine. Methionine analyses were made according to the method of McCarthy and Sullivan ('41). Cystine was determined by precipitating with the cuprous chloride reagent of Rossouw and Wilken-Jorden ('35) and determining the amount of sulfur removed from the solution.

Preliminary experiments indicated that a basal diet in which the only protein source was the isolated soybean protein could not support a good rate of growth, but that if methionine was added, good growth was obtained. Soybean protein autoclaved for 1 hour at 15 lbs. per square inch did not lead to better growth than did the uncooked protein. This fact indicated either that the heating process did not liberate the methionine present, or that practically all the methionine present was available. In the investigations of Klose and Almquist ('41), cystine was present in adequate amount, yet the percent gain was less than zero unless methionine was added. In the present studies, the percent gain per day was 1.2 for the chicks on the basal diet without any addition of sulfur amino acids, which indicated that the amount of methionine present was largely available, although definitely suboptimal.

Chicks were placed on a low-choline diet at hatching time and maintained on this diet for 16 to 21 days to deplete them of choline. This diet was a modification of that used by Jukes ('41), and consisted of water-washed casein 18, glucose [2] 50.5, gelatin 8, gum arabic 5, yeast [3] 9, mineral mixture 4.24, crude soybean oil 5, and sardine oil (400 D–3000 A) 0.3 gm. per 100 gm. The mineral mixture furnished the following to each 100 gm. of diet: tricalcium phosphate 2000 mg., dipotassium phosphate 500, potassium chloride 300, sodium chloride 100, and manganese 10, silicon 46, magnesium 48, aluminum 8, iron 14, copper 1, zinc 1, iodine 0.8 and cobalt 0.5. After the depletion period, the chicks were banded, weighed and segregated into groups of three or four chicks carefully selected for weight, gain and vigor. These chicks showed a high incidence of incipient perosis, but were not yet severely crippled by the deficiency.

The basal diet to which the various supplements were added consisted of isolated soybean protein 23, glucose [2] 52.8, cellulose [4] 5, calcium gluconate 8, mineral mixture as in the low-choline diet 4.24, cottonseed oil [5] 5, cod liver oil (U.S.P.) 1, 2-methyl-1, 4-naphthohydroquinone diphosphate (sodium salt) 0.001, thiamine 0.001, riboflavin 0.001, pyridoxine 0.001, nicotinic acid 0.005, calcium pantothenate (dl) 0.003, and a biotin source [6] 1 gm. per 100 gm. The amount of soybean protein used provided 20 gm. of protein per 100 gm. of diet; hence the methionine and cystine contents of the basal diet were 0.3% and 0.05%, respectively.

The chicks were fed the experimental diets for 6 days, during which time they were weighed daily. The supplements were commercial products with the exception of S-methylcysteine, which was prepared according to the method of Clarke and Inouye ('31–'32) as modified by Brand, et al. ('37).

TABLE 1

The relation of additions of sulfur-bearing amino acids to percent gain per day.

DIET NUMBER	SUPPLEMENTS ADDED TO THE BASAL DIET [1]				TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100-GM. DIET	NUMBER OF GROUPS	AVE. % GAIN PER DAY
	l-Cystine	dl-Homo-cystine	dl-Meth-ionine	Choline chloride			
1	...	...	...	...	...	2	1.2
2	0.20	...	...	...	1.67	3	1.8
3	0.40	...	...	...	3.34	2	1.9
4	0.60	...	...	...	5.01	1	2.0
5	...	0.23	...	...	1.72	1	1.8
6	...	0.45	...	...	3.36	1	1.5
7	...	...	0.25	...	1.68	1	3.7
8	...	...	0.50	...	3.36	1	4.2
9	...	...	1.00	...	6.72	1	4.3
10	0.20	0.23	...	...	3.39	2	2.0
11	0.20	0.45	...	...	5.03	1	1.6
12	0.20	...	0.50	...	5.03	1	4.4
13	...	...	...	0.20	...	1	1.1
14	0.20	...	...	0.20	1.67	2	4.0
15	0.30	...	...	0.30	2.50	1	4.0
16	...	0.23	...	0.20	1.72	2	3.8
17	...	...	0.50	0.20	3.31	1	5.3
18	0.20	0.23	...	0.20	3.39	1	5.1
19	0.20	0.45	...	0.20	5.03	2	6.4
20	0.20	...	0.25	0.20	3.35	1	5.2
21	0.20	...	0.50	0.05	5.03	1	6.0
22	0.20	...	0.50	0.20	5.03	1	6.0
23	0.20	...	0.70	0.20	6.37	1	6.3

[1] Expressed in percent added to diet.

TABLE 2

Experiments with S-methylcysteine and with l- and dl-methionine.

SUPPLEMENTS ADDED TO THE BASAL DIET [1]						TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100-GM. DIET	AVE % GAIN PER DAY
l-Cystine	dl-Homo-cystine	S-Methyl-cysteine	dl-Meth-ionine	l-Meth-ionine	Choline chloride		
...	0.23	0.23	...	...	...	1.72 [3]	1.7
...	0.23	...	...	...	...	1.72	1.8
...	...	0.22	...	...	0.20	1.63	1.7
0.20	...	...	...	...	0.20	1.67	4.0 [3]
...	...	...	0.25	...	...	1.68	3.7
...	...	...	...	0.25	...	1.68	3.8
0.20	...	...	0.25	...	0.20	3.35	5.2
0.20	...	...	...	0.25	0.20	3.35	5.3

[1] Expressed in percent added to diet.
[2] On basis of homocystine alone.
[3] Mean of two groups.

DISCUSSION

The data obtained from the various experiments are presented in tables 1 and 2, which show the relationships existing between the various supplements added and the average per cent gain per day. The latter quantity is calculated by the equation

$$\text{Ave. \% gain per day} = \frac{\text{Ave. gain} \times 100}{\text{Ave. wt. during expt.} \times \text{no. days on expt.}}$$

For non-depleted chicks of the same breed and of approximately the same age on a practical ration, the percent gain is about 7.5; the supplemented groups which attained the best rates of growth showed an average gain of 6.3%. This difference is not great in view of the synthetic nature of the diets.

Figure 1 has been plotted to show more clearly the relations between sulfur amino acids added and percent gain when choline is absent and present in the diet. In order to present
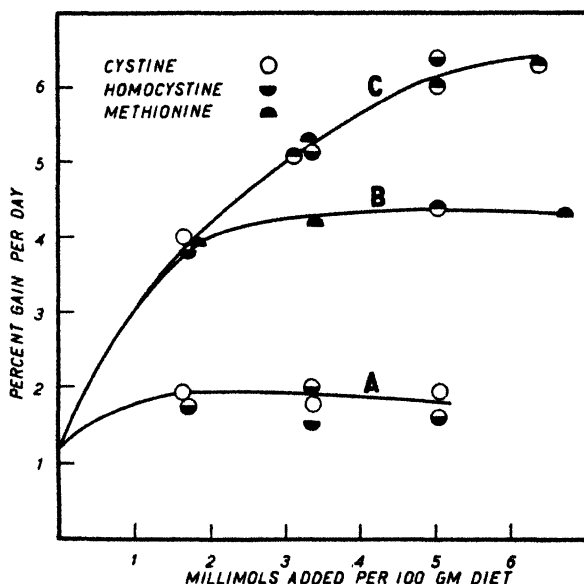
Fig. 1 The relation of added sulfur amino acids to the rate of growth. Curves A and B represented results obtained with diets to which no choline was added. The results represented by curve C were obtained with diets which contained 0.2% added choline chloride.

all data on a comparable basis, the total millimols of amino acids added have been used as abcissae. In the case of methionine, the actual number of millimols was used; with the double molecules of cystine and homocystine, twice the actual number was used.

No choline added

Up to approximately 2 millimols of added sulfur amino acids, the basal ration supplemented with methionine (fig. 1, curve B) supports a rate of growth equivalent to that shown by the groups receiving choline with homocystine or cystine (compare diet 7 with diets 14 and 16; also see fig. 1, curve C). Since the basal diet is deficient in both cystine and methionine, but contains an appreciable amount of the latter, it not surprising that, within limits, the form of amino acid added is immaterial to the growth rate. At higher amino acid levels the deficiency of choline becomes a growth-limiting influence and the percent gain increases very slowly to only 4.4 at 5 or more millimols of added sulfur amino acids, while the percent gain of the pens receiving choline in addition is about 6.3 (compare diet 12 with diets 19 and 22). These data confirm reports (Jukes, '41; Record and Bethke, '42) on the irreplaceability of choline by methionine. Curve A of figure 1 clearly shows that choline-depleted chicks cannot effectively utilize homocystine in lieu of methionine. It also indicates that homocystine is equivalent to cystine when no choline is added to the diet.

It may be merely a coincidence that the growth rates with cystine and with homocystine are practically identical; this may be the result of a partial methylation of homocysteine,[7] which is not enough to bring the gains close to those supported by methionine alone. In this event, however, it would seem that the growth effects of cystine and homocystine should be additive to some extent, which is evidently not the case. The most probable interpretation is that homocystine is converted

[7] It is assumed that the reduction of homocystine to homocysteine proceeds without interference in the choline-depleted chick. This reduction would seem to be a necessary step in the indicated conversion of homocystine to cystine, as well as to methionine.

to cystine, as indicated in the rat (Binkley and du Vigneaud, '42), and the amount of either one added is sufficient to provide all the cystine that can be utilized in the presence of the residual amount of methionine in the basal diet. This interpretation further implies that both *d* and *l* isomers of homocystine are utilized by the chick as sources of cystine when fed in a choline-deficient diet. It is realized that proof of such dual conversion is yet to be obtained.

Choline added

When choline is added to the basal diet (curve C), the same curvilinear relationship as in curve B holds between the percent gain and the added sulfur amino acids up to 2 millimols. In this range, cystine plus choline, or homocystine plus choline are equivalent to methionine without choline (compare diets 14 and 16 with diet 7).

In contrast to the curve in the absence of choline, the growth values continue to rise steeply where more than 2 millimols of sulfur amino acid are needed. Like methionine, homocystine now exerts a growth effect which is additive to that of cystine (compare diet 18 with diet 20 and diet 19 with diet 22).

S-Methylcysteine

Homocystine was fed in the absence of choline but in the presence of S-methylcysteine to demonstrate any possible methylating action of this compound (table 2). There was no difference between the group which was fed S-methylcysteine and the control group. These results are similar to those obtained with the rat (Welch, '41; Moyer and du Vigneaud, '42).

An experiment which was designed to test the cystine-replacing power of the methyl compound showed that it is ineffective in this capacity also. These results are in agreement with those obtained by Block and Jackson ('32) in experiments with the rat.

Natural and synthetic methionine

Two sets of experiments in which *l*- and *dl*-methionine were compared (table 2) showed these forms to be equally active. In the first experiment the *l* and *dl* forms were compared in the presence of 0.2% choline chloride and 0.2% cystine; in the second experiment the two forms alone were added. Both of these sets of data fit very well into the curves plotted in figure 1.

These results with chicks confirm growth experiments with rats (Jackson and Block, '33) which indicate that *d*- and *l*-methionine are equivalent. There is some discrepancy between this work and that of Bennett ('39), who reported that "*dl*-methionine is somewhat less readily utilized for growth than the *l* form." Balance experiments with dogs (Stekol, '35) disclosed no differences between the *l* and *dl* forms.

Sulfur amino acid requirements of the chick

In view of the evident equivalence of the *l* and *dl* forms, it seems permissible to conclude that former estimates of the methionine requirement of the chick, based partially on supplementation with the *dl* form, are valid. A summary of such estimates is given in table 3. These would indicate that the total cystine plus methionine requirement is approximately 1.0 to 1.1% (5.0 millimols per 100 gm.) and may be met by varying proportions of these amino acids, except that the minimum methionine level is approximately 0.5 to 0.6%.

TABLE 3

Combinations of cystine and methionine in the diet of the chick which permit maximal gains

CYSTINE	METHIONINE	SOURCE
percent	percent	
.25	.80	Present report
.55	.54	Almquist, et al., '42
.60	.55	Almquist and Jukes, '42
.30 [1]	60 [1]	Briggs, et al., '42

[1] Possibly small additional amounts present.

SUMMARY

With the use of a diet deficient in methionine, cystine and choline, the relationships between the sulfur-bearing amino acids and choline in the chick have been further investigated. The following conclusions have been drawn:

1. The choline-depleted chick cannot appreciably utilize homocystine in place of methionine.

2. Homocystine can be utilized in lieu of cystine by choline-depleted chicks.

3. *dl*-Methionine is equivalent to *l*-methionine for growth, whether or not choline is present in the diet.

4. S-Methylcysteine can neither assist in the utilization of homocystine nor replace cystine in the diet of the chick.

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THE COMPARATIVE TOXICITY OF CALCIFEROL, A.T. 10, AND COD LIVER OIL CONCENTRATE FOR CHICKS

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In the past few years the chicken has become a popular biological tool for studying the metabolic influence of antirachitic substances. There appears, however, to be little information concerning the response of the chick to these agents if administered in massive doses.

Investigations with mammals have generally concluded that massive doses of vitamin D_2 are toxic, demonstrating increased serum calcium levels accompanied by bone dissolution. Not all reports substantiate one or the other or both of these claims. The less frequently studied irradiation product of ergosterol, dihydrotachysterol, commonly referred to as A.T. 10, has been shown (Correll and Wise, '42) to have a low antirachitic potency. The literature indicates that for mammals it is hypercalcemic and toxic. Its effect in large doses on phosphatase activity and bone structure has not been thoroughly explored. Some investigators consider this sterol a parathyroid hormone substitute and question its relation to vitamin D metabolism. Studies available on toxic levels of cod liver oil usually involve oils of relatively low vitamin D potency and stress the histological effect on certain specific organs rather than enzyme and mineral metabolism. The field of antirachitic sterol metabolism has been admirably reviewed by Reed, Struck, and Steck ('39). McLean ('41) has published a later survey concerning A.T. 10.

It seemed likely that the use of the chick in studies involving massive doses of these antirachitic agents might add to our knowledge concerning their mode of action. By following the serum calcium, phosphorus and phosphatase values as well as bone ash content in the experiments herein reported, we hoped to learn in what ranges vitamin D_2, A.T. 10 and vitamin D from cod liver oil were toxic for chicks and if in massive doses they would produce hypercalcemia with bone dissolution.

The data were also expected to reveal whether A.T. 10 would augment serum phosphatase levels in the chick, as parathyroid hormone reportedly does in mammals (Cantarow, Brundage and Housel, '37; Gutman, Tyson and Gutman, '36), or whether it would, like vitamin D, maintain low normal concentrations.

Finally, it has been our experience as previously reported (Correll and Wise, '38) that rachitic birds with characteristic low bone ash demonstrate a greatly elevated serum phosphatase concentration. If massive doses of any of these substances resulted in bone demineralization as indicated by low bone ash, we were anxious to ascertain whether serum phosphatase values would remain low or would rise as they always do in rachitic subjects with faulty bone composition. The answer obtained might be helpful in the solution of the problem as to whether the greatly increased phosphatase content of plasma in rickets and certain bone diseases is the cause or effect of the recognized mineral insufficiency.

<center>EXPERIMENTAL</center>

Single comb white Leghorn chickens when 1 day old were divided into groups of twenty and placed on a rickets-producing basal ration consisting of ground yellow maize 57 parts, wheat flour middlings 25, crude casein 12, calcium carbonate 1, calcium phosphate 1, iodized salt 1, brewer's yeast 2, and maize oil 1. Analysis revealed the mixture to contain 1.0% calcium and 0.7% phosphorus. Some assay groups at the higher levels, as indicated in table 1, included fewer birds

because the quantity of high potency material available was limited.

The sterols used were so diluted when possible (exceptions are noted in the table) that 0.1 ml. contained the desired daily dose. This was administered to each chick per os with a 1-ml. tuberculin syringe carrying an 18-gauge blunted needle. Injections were made daily, except Sunday, over a period of 21 days at which time those that survived were sacrificed. Material for analysis was taken from each remaining bird up to fifteen from a group. The technique for obtaining pooled blood and bone samples and determining bone ash was essentially as described in a prior report (Correll and Wise, '38). Serum calcium values were ascertained by the method of Clark and Collip ('25), phosphorus and phosphatase according to Bodansky ('33). Judgment as to toxic doses was based on the gross appearance of the birds, failure to grow as shown by weekly weighings, and the number of chicks that died towards the end of the experimental period.

The vitamin D unitage, as indicated by U.S.P. XII rat assays conducted in this laboratory, served as a basis for deciding at what dosages the substances under investigation were administered to the chicks. The lower levels of vitamin D from cod liver oil were prepared from an oil having a potency of 270 I.U. per gram; the massive doses from a concentrate assaying 66,000 I.U. per gram. The smaller doses of vitamin D_2 were dilutions of a solution of irradiated ergosterol with an activity of 10,000 I.U. per gram; massive doses were made up from crystalline calciferol (m.p. 116°C.) assaying 40,000,-000 I.U. per gram. The dihydrotachysterol dilutions originated from a commercial product [1] which showed an antirachitic potency of 30,000 I.U. per gram of sterol content.

Approximately ten separate experiments were conducted. Each consisted of ten groups including a negative and positive control group. The negative controls received only the basal ration. As positive controls we used chicks that were fed ad

lib. throughout the experimental period the basal diet in which had been incorporated into the maize oil portion sufficient cod liver oil to furnish 20 I.U. of vitamin D in each 100 gm. of ration. Previous feeding experiments have shown us that such a supplement in the basal diet is about minimal for normal mineral, phosphatase, and bone ash response in chicks of this age and hence could be used as a standard of comparison to determine the adequacy of an antirachitic substance administered at different levels and by any route. Past records of food consumption on groups similar to these positive controls were only obtained for the group as a whole but they allowed an estimation of the daily intake of vitamin D per chick to be around 2 to 3 I.U. This estimation served as a basis for establishing more accurately the individual daily minimum requirement.

RESULTS AND DISCUSSION

Since the minimum daily prophylactic dose of the sterols to be studied necessary to maintain normal serum calcium, phosphorus, and phosphatase values, and bone ash content in chicks, was not known, our first objective was to establish these values. We then proceeded to determine toxic levels and observe the response to toxic and subtoxic doses.

The data obtained are presented in condensed form in table 1. The values recorded for the controls are averages for such groups from all the experiments. The physiological effects on the chick of the sterols listed were studied several times at numerous levels. The groups submitted in table 1 for comparison with the control values represent typical responses to the doses indicated.

It can be seen in experiment 1 that normal serum calcium, phosphorus, and phosphatase values, and adequate bone ossification, were attained upon the daily administration of 3 I.U. of vitamin D from cod liver oil (group 555). Toxicity was evident at a level around 15,000 I.U. of vitamin D (group 556) from this particular concentrate, or at 5000 times the minimum daily dose. Even the massive doses produced no hypercalcemic

TABLE 1

Serum calcium, phosphorus, and phosphatase (per 100 ml. of serum), and bone ash values of chicks given massive doses of several sterols.

GROUP NO.	DAILY DOSE Weight	DAILY DOSE I.U.	FINAL WEIGHT	INORGANIC CALCIUM	INORGANIC PHOSPHORUS	PHOSPHATASE	BONE ASH	COMMENTS
			gm.	mg.	mg.	units [2]	%	
				Controls				
Negative	0	.....	133	6.8	5.5	131	36.2	
Positive	20 I.U./100 gm.[1]	.....	152	10.4	5.9	44	47.0	
			Experiment 1. Vitamin D from cod liver oil					
501	3.7 mg.	1	149	7.9	5.5	103	39.9	
555	11.1 mg.	3	148	10.0	6.0	38	47.1	
506	15.2 mg.	1000	151	10.5	5.8	38	48.0	
508	152 mg. 0.2 ml.	10,000	142	10.2	5.4	35	49.0	
556	228 mg. 0.3 ml.	15,000	109	10.1	5.9	27	50.0	Two birds died. Toxic level.
558	380 mg. 0.5 ml.	25,000	91	10.3	5.5	28	49.0	Ten birds died.
			Experiment 2. Calciferol					
477	0.25 µg.	10	144	8.3	4.6	90	40.0	
536	2.5 µg.	100	142	10.5	5.3	47	46.9	
580	25 µg.	1,000	136	10.7	8.4	54	47.3	
582	0.25 mg.	10,000	137	11.6	8.2	33	49.0	
544	2.5 mg.	100,000	137	13.0	4.2	29	44.5	Five birds; all lived. Five birds; four died.
573	3.1 mg.	125,000	...	...	...	...	...	Toxic level.
			Experiment 3. Dihydrotachysterol (A.T. 10)					
494	8.3 µg.	0.25	104	8.9	5.5	75	39.2	
546	16.6 µg.	0.5	154	10.6	5.0	44	49.7	
547	33.3 µg.	1	147	11.2	6.0	33	48.3	
548	66.6 µg.	2	160	12.4	4.8	45	47.2	
562	166.5 µg. 0.2 ml.	5	94	13.8	4.5	29	43.7	Ten birds; six died. Toxic level.
563	249.8 µg. 0.2 ml.	7.5	63	14.5	...	< 27	38.6	Eleven birds; nine died.

[1] For explanation see text under Experimental.   [2] Bodansky, '33.

effect nor bone dissolution. Antithetically, as the dose tended to be toxic, bone calcification if anything became more dense (groups 556, 558). Serum phosphatase concentrations remained within the normal range or lower.

The toxicity of such a concentrate for chicks, when given at levels of better than 200 mg. per day, may well be due to some constituent unrelated to the antirachitic entity. However, the data herein presented show that extremely high doses of vitamin D from cod liver oil in the chick did not cause a deviation from normal of the serum calcium, phosphorus or phosphatase levels, or bone ash content.

In experiment 2 the daily administration of 100 I.U. of vitamin D_2 (group 536), yielded mineral, enzyme and bone values comparable to the positive controls. Doses around 100,000 I.U. per day (group 544), or 1,000 times the minimum requirement, approached a toxic range. With calciferol in this quantity of 2.5 mg. daily, representing the enormous dose of 100,000 I.U. of vitamin D_2, there was a slight rise in serum calcium and indications that the mineral content of the bone was somewhat depleted, but the serum phosphatase remained low. Small additional increases in the daily dose (group 573) were so toxic as to be fatal for the birds before the end of the experimental period.

A.T. 10 (experiment 3) demonstrated normal responses when 0.5 I.U. were given daily (group 546), and toxic manifestations at ten times this level or 5 I.U. per day (groups 562). The fact that 16.6 µg., equivalent to only 0.5 I.U. of antirachitic activity, was sufficient to yield mineral responses in the chick comparable to those obtained by 3 I.U. from cod liver oil (group 555) or 100 I.U. of vitamin D_2 (group 536) again emphasizes (Correll and Wise, '42) the remarkable difference in the antirachitic substance present in A.T. 10.

As the daily level of A.T. 10 was increased towards the definitely toxic doses (groups 562, 563) the serum calcium became progressively higher while bone ash values receded. It will be noted, however, that with the obvious bone dissolu-

tion, the serum phosphatase concentrations never rose; in fact, they actually went below the positive controls.

Thus in the chick this interesting sterol of plant origin demonstrates a greater antirachitic efficiency on a rat unit basis than the vitamin D from cod liver oil. In contrast, however, its toxic dose is only about ten times its minimum protective level and this is accompanied by a pronounced increase in serum calcium concentration with bone decalcification. The hypercalcemia resembles the action of parathyroid hormone but no increase was found in the serum phosphatase as has been reported for parathyroid hormone in other species.

In no instance, where there was bone dissolution after administration of massive doses of an antirachitic agent, was there any rise in serum phosphatase. This suggests that the increased enzyme values observed in rickets is probably not a direct result of a physical weakening of the bone structure per se.

SUMMARY

1. Vitamin D from cod liver oil at 3 I.U. (11.1 mg.) daily affected normal mineral responses in chicks. When given at high levels no deviation from normal was seen with this particular concentrate, in the serum calcium, phosphorus, and phosphatase or the bone ash values; toxic manifestations were noted at about 5,000 times the minimal dose. It is recognized that the observed toxicity for the concentrate may be due to something besides its vitamin D activity.

2. Calciferol at 100 I.U. (2.5 μg.) yielded normal values; toxicity was apparent at about 1,000 times this dose. In enormous doses a slight hypercalcemia and bone dissolution was found. Phosphatase values remained low at these high levels.

3. Only 0.5 I.U. (16.6 μg.) of antirachitic activity was required daily from A.T. 10 to produce normal mineral metabolism in the chick. This emphasizes the individuality of the antirachitic substance present in A.T. 10 when compared with the other rickets-preventing sterols studied. A.T. 10 was

toxic for chicks in these experiments at levels around ten times the minimal daily requirement. In the massive doses there was a hypercalcemia and bone dissolution.

4. A.T. 10, like vitamin D, will prevent rickets in chicks. Its effect in increasing serum calcium and lowering bone ash appears to be of a degree that resembles parathyroid hormone. However, even in massive doses it caused no increase in serum phosphatase. Thus this sterol preparation influences calcium metabolism in a manner which resembles both vitamin D and parathyroid hormone.

5. In no case where massive doses of sterols resulted in bone ash depletion, was there observed any increase in serum phosphatase concentration.

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CAROTENE REQUIREMENTS FOR THE MAINTENANCE OF A NORMAL SPINAL FLUID PRESSURE IN DAIRY CALVES [1]

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The minimum requirement of calves for carotene has been the subject of several recent reports. Guilbert and co-workers ('37) reported a minimum requirement for growing calves of 30 µg. of carotene per kilogram of body weight using nyctalopia as a criterion. This requirement they believed to be the physiological minimum. Moore ('39c) confirmed the figure given by Guilbert using nyctalopia as a criterion but stated that this amount was not sufficient to prevent papilledema in calves. Therefore, the 30 µg. level would be below the physiological minimum. Boyer, Phillips, and co-workers ('42a) found 75 µg. per kilogram for Holstein yearlings and 125 µg. per kilogram for Guernsey yearlings necessary to maintain an adequate plasma vitamin A level. Keener and associates ('42) found that 12 µg. per pound (27 per kilogram) met the minimum requirements at an environmental temperature of 50° to 70°F. However, these investigators state that during severe winter weather the minimum requirement may be more than twice as great as during warm weather. Ophthalmoscopic observations, blood vitamin A levels, and histopathological alterations were used as criteria.

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Moore ('39b), Wetzel and Moore ('40) reported papilledema in calves fed vitamin A deficient rations. Also, it was demonstrated that the papilledema could be prevented by feeding crystalline carotene dissolved in cottonseed oil. Papilledema is a result of an increase in pressure of the cerebrospinal fluid. Moore and Sykes ('40) ('41) found abnormally high pressures of the spinal fluid in calves fed vitamin A deficient rations which could be alleviated by feeding crystalline carotene dissolved in cottonseed oil.

Because of these results it was believed that periodic measurements of the pressure of the spinal fluid might be used as a criterion for the adequacy or inadequacy of various intakes of carotene. Data collected from such experiments are herein reported.

<div align="center">EXPERIMENTAL</div>

Two groups of calves were used in this experiment. The data on the first group were obtained at the dairy section of the Michigan Agricultural Experiment Station, East Lansing, while the data on the second group were collected at the dairy section of the Maryland Agricultural Experiment Station, College Park. Pertinent differences in the management, experimental procedures, and results will be alluded to throughout the paper.

Calves 3 months of age were placed on a basal ration low in carotene for a 30-day depletion period, after which carotene in the form of dehydrated alfalfa leaf meal was added at various levels of intake. The basal ration consisted of 240 lbs. of ground barley, 180 lbs. of rolled oats, 180 lbs. of wheat bran, 60 lbs. of linseed meal, and 8 lbs. of salt. Ten pounds of skimmed milk per calf was fed to group I while 1 lb. of skimmed milk powder was fed daily to group II. Viosterol was fed as a source of vitamin D during winter months. Wood shavings were used as bedding.

The calves in group I were weighed every 10 days and those in group II, weekly, at which time adjustments were

made on the intake of carotene which was fed according to body weight. The small amount of carotene in the basal ration was taken into account.

The carotene content of the alfalfa leaf meal was determined chromatographically by a previous published procedure (Moore, '40 and Moore and Ely, '41). Weekly carotene determinations on the blood plasma of the first group were made by a previously published method (Moore, '39a). Plasma vitamin A and carotene on the second group were determined by a modification of the previous carotene method as suggested by Kimble ('39). The determinations were made with a photoelectric spectrophotometer with a slit width of 5 millimicrons. A wave length of 455 millimicrons was used for carotene and 620 for the antimony trichloride reaction for vitamin A. Plasma ascorbic acid determinations for group II were made every 2 weeks by the macro method of Mindlin and Butler ('38). A wave length of 510 millimicrons was used to determine the fading of the dye.

The method for making the spinal puncture and the measurement of the spinal fluid pressure has previously been described by Sykes and Moore ('42). In the second group of calves a small bore glass tube replaced the water manometer which obviated releveling with changes in the position of the head or body of the calves. The glass tube was connected to the needle by means of a short piece of rubber tubing and the height to which the spinal fluid rose in the tube was noted. Ophthalmoscopic observations were made periodically.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results obtained with the two groups of calves. These data have been summarized for 30-day periods. Since samples of blood for vitamin A and carotene determinations were drawn each week, the values shown are the average of two to five determinations. The plasma ascorbic acid values are the average of two determinations.

TABLE 1

Results in 30-day periods with Ayrshire and Holstein calves in group I.

Days	INTAKE 46 μG. CAROTENE PER KG. C 440 AYR MALE 12-5-39 [1]		INTAKE 62 μG. CAROTENE PER KG. C 442 HOL MALE 2-10-40 [1]		INTAKE 62 μG CAROTENE PER KG. C 439 AYR MALE 12-5-39 [1]		INTAKE 68 μG. CAROTENE PER KG. C 468 HOL MALE 5-26-41 [1]		INTAKE 77 μG. CAROTENE PER KG. C 456 HOL MALE 11-8-40 [1]		INTAKE 77 μG. CAROTENE PER KG. C 457 HOL MALE 11-27-40 [1]		INTAKE 128 μG. CAROTENE PER KG. C 441 HOL MALE 12-5-40 [1]	
	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O	Plasma carotene μg per 100 ml	Spinal fluid pressure m.m. H_2O
120–150	19	105	16	110	12	90	…	90	37	80	31	80	30	120
150–180	37	115	21	120	27	125	…	…	35	95	42	85	59	…
180–210	45	120	30	160	43	110	…	130	43	90	41	90	85	105
210–240	50	165	35	200	66	135	…	…	41	110	43	95	83	85
240–270	59	170	40	190	98	…	93	120	48	85	40	90	78	95
270–300	73	165	58	240	92	…	80	115	42	90	27	120	83	…
300–330	47	200	40	200	87	…	…	…	26	105	39	100	71	120
330–360	75	165	32	…	80	160	…	…	36	105	54	…	90	110
360–390	41	…	32	210	53	…	121	120	82	…	…	90	90	…
390–420	59	210	35	180	72	190	82	120	…	90	…	…	82	120
420–450	84	220	…	…	93	190								
450–480	…	…	…	…	100	190								

[1] Date when 120 days of age.

TABLE 2

Spinal fluid pressure and plasma vitamin A, carotene and ascorbic acid in calves of group II in 30-day periods

Left half

DATE	AGE	Vitamin A	Carotene	Ascorbic acid	SPINAL FLUID PRESSURE
	days	*µg /100 ml*			*mm. H_2O*
Intake 44 µg. carotene/kg. 474 Holstein Male					
7- 5-42	120	2	4	..	...
8- 4-42	150	5	8	.18	85
9- 3-42	180	8	17	.30	75
10- 3-42	210	8	18	.36	75
11- 2-42	240	7	24	.27	125
12- 2-42	270	7	29	.32	130
1- 1-43	300	7	18	.28	180
1 30-43	330	7	22	.17	210
3- 1-43	360	.	..	..	...
Intake 62 µg. carotene/kg 463 Ayrshire Male					
2 16-42	120	12	6	..	95
3 18-42	150	13	36	..	...
4 17-42	180	14	51	..	130
5 17-42	210	14	46	.34	110
6-16-42	240	14	38	.29	85
7-16-42	270	15	38	.27	85
8 15-42	300	15	47	.27	75
9 14-42	330	12	45	.31	90
10 14-42	360	12	55	.25	120
11-13-42	390	14	34	.18	...
12-13-42	420	12	47	.29	155
1-12 43	450	11	49	..	195
470 Holstein Female					
6-15-42	120	15	24	.33	70
7-14-42	150	16	36	.29	70
8-13-42	180	14	57	.32	70
9-12-42	210	14	52	.28	110
10-12-42	240	11	53	.25	115
11-11-42	270	12	51	.30	140
12-11-42	300	9	47	.31	170
1-10-43	330	8	56	.23	230
Intake 70 µg carotene/kg. 466 Holstein Male					
4- 4-42	120	10	25	..	85
5- 4-42	150	9	34	.25	...
6- 3-42	180	10	39	.27	70
7- 3-42	210	8	24	.26	...
8- 2-42	240	11	30	.31	70
9- 1-42	270	10	38	.37	70
10- 1-42	300	7	59	.31	70
10-30-42	330	8	60	.35	80
11-29-42	360	8	43	.35	95
12-29-42	390	7	52	.32	85

Right half

DATE	AGE	Vitamin A	Carotene	Ascorbic acid	SPINAL FLUID PRESSURE
	days	*µg /100 ml.*			*mm. H_2O*
Intake 62 µg. carotene/kg. 472 Guernsey Male					
6-28-42	120	9	24	..	...
7-28-42	150	13	38	.34	115
8-27-42	180	10	49	.31	75
9-26-42	210	7	36	.28	120
10-26-42	240	10	32	.33	...
11-25-42	270	10	36	.31	160
12-25-42	300	8	33	.20	190
1-24-43	330	6	39	..	160
Intake 66 µg. carotene/kg. 464 Ayrshire Male					
4-12-42	120	14	40	..	65
5-12-42	150	15	33	.28	65
6 11-42	180	13	41	.28	70
7-11-42	210	14	40	.30	...
8 10-42	240	12	63	.34	70
9- 9-42	270	10	46	.26	80
10- 8-42	300	10	47	.50	75
11- 7-42	330	11	49	.25	105
12- 7-42	360	8	37	.27	115
1- 6-43	390	11	59	.23	85
2- 5-43	420	11	67	..	...
471 Ayrshire Male					
6-15-42	120	7	11	.27	70
7-15-42	150	6	7	.27	75
8-14-42	180	7	11	.20	80
9-13-42	210	9	10	.24	75
10-13-42	240	11	20	.38	70
11-12-42	270	7	34	.30	100
12-12-42	300	7	34	.27	100
1-11-43	330	5	30	.16	95
Intake 75 µg. carotene/kg. 467 Holstein, Female					
5-17-42	120	8	20	..	...
6-16-42	150	11	27	.23	65
7-16-42	180	11	31	.29	65
8-15-42	210	8	34	.23	115
9-14-42	240	9	27	.20	85
10-14-42	270	11	52	.22	75
11-13-42	300	10	47	.36	145
12-13-42	330	7	55	.22	115
1-12-43	360	7	66	.28	110

The results show that an intake of 62 µg. of carotene per kilogram was not sufficient to prevent an increased spinal fluid pressure. Animals C442 and C439 of group I and 463, 470, and 472 of group II demonstrate this fact. On the other hand, an intake of 66–68 µg. was sufficient to maintain a normal spinal fluid pressure as shown by animals C468 of group I and 464 and 471 of group II. It would seem, therefore, that the measurement of the spinal fluid pressure is an exceedingly critical measure for detecting small differences in carotene intake. It appears that the minimum requirement is about 66 µg. per kilogram of body weight. These requirements are a little more than double those reported by Guilbert and associates ('37) and confirmed by Moore ('39c) where nyctalopia was used as a criterion. The differences noted are probably due to the different criteria used in this study as compared to the former studies, although environmental factors may be partly responsible. Moore ('39c) has shown previously that papilledema and presumably increased spinal fluid pressure developed in calves in which nyctalopia was not present.

It should be kept in mind that these requirements are for Holstein and Ayrshire calves from 4 months to 12 to 14 months of age. It is possible that calves from birth to 4 months of age have a higher requirement, and experiments reported by Converse and Meigs ('39) indicate that this may be true. The work by Kuhlman and Gallup ('40) shows a requirement of 40 µg. per pound (88 per kilogram) is necessary for proper reproduction.

The results further show the calves in group I receiving 62 µg. or less developed pressure during the early spring months so that they went into the summer months with high pressures. The pressures showed no tendency to decrease during the summer months. On the other hand, the calves in group II receiving 62 µg. or less did not develop the increased spinal fluid pressure during the summer months. The increase in pressure was delayed until the winter months. These results might at first glance appear contradictory unless certain facts of climate and management are taken into con-

sideration. For instance the average July temperature at East Lansing, Michigan, is 71.1°F., while that for College Park, Maryland, is 75.9°F., (Yearbook, '41). Furthermore, the humidity during the summer months at East Lansing is lower than at College Park making the effective temperature lower. A possible further contributing factor is the fact that at East Lansing the calves of group I were always placed in the barn by 10:30 A. M. or before, while at College Park the calves in group II were left to considerable exposure to sunshine throughout the day. Under these conditions high body temperatures, 104–105°F. were often recorded for calves in group II in the summer months.

It is not possible with the present data to compare the requirements of the calves at East Lansing during the summer months with those of the calves at College Park for a similar period nor is it possible to definitely ascribe the differences noted above to environmental temperature. However, these data do suggest that where spinal fluid pressure is used as a criterion, the requirement is higher during the winter months. The data are somewhat more conclusive in that they tend to confirm the excellent observations of Keener and associates ('42). While Keener did not make spinal fluid pressure measurements, he did make ophthalmoscopic observations for papilledema which is a result of an increased spinal fluid pressure. Keener also observed lower plasma vitamin A values during the winter months. The results in these experiments confirm that observation.

A study of the plasma vitamin A values does not show any close correlation between carotene intake and these plasma vitamin A values except where wide variations of intake are considered. As a matter of fact the vitamin A values for calves 470 and 463 receiving an intake of 62 µg. of carotene are higher than for calves 471 receiving 66 µg., 466 receiving 72 µg., and 467 receiving a 75 µg. intake. Within these narrow limits of intake therefore vitamin A values of the blood plasma are of little value as a criterion of adequacy or inadequacy. This fact seems to be fairly well established since these calves

were maintained on the same level of intake over a period of 10 to 12 months with blood samples being drawn every week. There would seem to be individual characteristics of each animal which determine the level of vitamin A in the blood. A study of the data presented by Davis and Madsen ('41) appears to indicate considerable variation. The same conclusion can be drawn from a study of the carotene values. It should be emphasized that at times there are considerable weekly and periodic variations in the vitamin A and carotene values with these levels of intake. When the intakes are cut down to 16 µg. or below, the carotene variations are much less pronounced as shown by previously published data (Moore, '39c).

It is not the purpose of this discussion to leave the impression that determinations of plasma vitamin A values are of no value. From a practical standpoint, if the limitations are understood, they are quite valuable. The values for animal 474 receiving only 44 µg. are apparently lower than for the other animals. Therefore, one could probably tell the difference between an intake of 44 and 75 µg. but not between 62 and 75.

Among the seven calves which received less than 66 µg. of carotene per kilogram of body weight and which showed an increased spinal fluid pressure, four developed papilledema. The nerve heads of the other three calves apparently remained normal. Two factors working together probably account for this variation. First, most of these seven calves received carotene just below the minimum rate so that a marked increase in spinal pressure did not develop. The higher the spinal pressure the more marked is the papilledema. Secondly, there are some age and individual variations in cattle of the intraocular tension (unpublished data). A high intraocular tension would prevent the papilledema from developing especially where the spinal fluid pressure was not greatly elevated.

Boyer and co-workers ('42b) have pointed out a possible relationship between vitamin A and C and the pressure of the spinal fluid. Consequently plasma ascorbic acid determinations

were made on the calves of group II. No particular correlation between plasma ascorbic acid levels and increased spinal fluid pressure is noted. However, since the pressures are not high and since the variations of carotene intake are small, the authors wish to withhold further comment until more critical experiments now in progress have been completed.

SUMMARY

1. The results of these experiments indicate that measurement of the spinal fluid pressure of calves is a fairly critical index of adequacy or inadequacy of carotene intake.

2. An intake of 66 µg. per kilogram of body weight during the winter months is about the minimum requirement for carotene for Holstein and Ayrshire calves when spinal fluid pressure is used as a criterion.

3. Because of individual characteristics, plasma vitamin A or carotene values will not distinguish between variations of carotene intake of 62 to 75 µg. per kilogram of body weight.

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YEARBOOK OF AGRICULTURE 1941 Climate and man.

INFLUENCE OF INCREASING DOSES OF THIAMINE AND RIBOFLAVIN ON EFFICIENCY OF THEIR UTILIZATION [1]

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Knowledge of the relation of size of dose to efficiency of utilization of the B vitamins and what happens to excessive doses is important for many reasons. It should throw some light on the question of the true physiological minimum. It should also indicate whether the body's disposition of excessive doses involves merely the excretion of amounts that are greater than can be stored, or whether this excess is actually destroyed by the tissues, a fact that would serve to explain in part at least why some patients have been found to require unusually large amounts of thiamine in order to show clinical improvement.

A review of the literature disclosed that Leong ('37) made a study of the metabolism of vitamin B_1 and investigated the maximum storage of that vitamin in rats. He used the bradycardia method of Drury and associates ('30), which, compared with chemical procedures now available, must be considered qualitative rather than quantitative. This investigator concluded that the fecal excretion of vitamin B_1 in rats, apart from the small constant output attributable to bacterial synthesis, was insignificant with intakes of less than 150 µg. Incomplete absorption of the vitamin occurred when this level of intake was exceeded. When 750 µg. of vitamin B_1 were injected sub-

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·cutaneously, 75% of this dose was excreted in the urine. Maximum storage was obtained when the intake of vitamin B_1 was 90 µg. per day, a further increase above this level giving rise to no appreciable accumulation of tissue reserves.

The qualitative nature of Leong's procedures and the use of a crude product of thiamine invited a further investigation of this problem, using quantitative chemical methods and pure crystalline riboflavin as well as thiamine.

In a recent communication (Sure and Ford, '42) dealing with thiamine and riboflavin interrelationships in metabolism, a technique was described for determining these vitamins in rat urine and feces, using fluorophotometric procedures. For thiamine we employed the method of Hennessy and Cerecedo ('39), as modified by the research laboratories of Merck and Company ('41). For the riboflavin determination in feces, tissues, and glands we followed the method of Conner and Straub ('41). For the riboflavin analysis of urine we used our modification of the method of Hodson and Norris ('39) for determining the riboflavin content of foodstuffs, the details of which are described elsewhere (Sure and Ford, '42).

The early results obtained on the riboflavin content of rat feces were very erratic, the weekly fecal excretion frequently being twice or more than the total intake. The high figures were most probably due to bacterial synthesis (Wildemann, '41; Mitchell and Isbell, '42). Bacterial synthesis in the rat feces were finally reduced to much smaller proportions by collecting the feces under petroleum ether, the solvent used in the next step for removing fats previous to analysis. On the whole, however, the quantitative chemical methods for the determination of thiamine and riboflavin in rat urine and feces seemed satisfactory enough to warrant efforts being made to obtain balance sheets on the intake and output of graduated increased doses from small to massive amounts, in order to determine the efficiency of utilization of these vitamins in the animal organism. The results of this study are submitted in tables 1 to 6 inclusive.

EXPERIMENTAL

Thiamine and riboflavin balance experiments were carried out on twenty-four male albino rats, 36 days of age, and weighing from 80 to 100 gm. They were fed ad libitum a purified ration of the following composition: Casein,[2] 18; agar-agar, 2; Sure's salts no. 1 (Sure, '41), 4; butter fat, 10; dextrose, 66. This diet was supplemented by daily allowances of 20 µg. pyridoxine, 6 mg. choline chloride, and 200 µg. calcium pantothenate. The thiamine and riboflavin were administered in daily doses of 5, 10, 20, 50, 100, and 1,000 µg. The metabolism experiments were carried out in groups of four. In each group two animals received the same thiamine and riboflavin doses, one was denied thiamine, and another was not given any riboflavin. Each group, therefore, had a negative thiamine and a negative riboflavin control, which furnished information on the amounts of these vitamins contributed by the body tissues in the urinary and fecal excretions. The first three groups were given daily doses of 5, 100, and 1,000 µg. and the metabolism experiments were conducted for 26 days. However, since it was found that one-half that period provided sufficient evidence for calculation on efficiency of utilization, the balance studies on 10, 20, and 50 µg. were continued for only 13 days.[3] The thiamine and riboflavin determinations in the urine were carried out 5 days a week. When 48-hour samples were run the urine was stored in a refrigerator, preserved with toluol and kept acid at a pH of 4 to 4.5. The feces, collected in bottles covered with petroleum ether, were analyzed once weekly. The urine of the animals receiving the high doses had to be considerably diluted, so that the readings in the fluorophotometer were approximately 1 µg. Of course, the negative controls excreted very small amounts of both thiamine and riboflavin, in many in-

[2] Secured from the Borden Company, New York under the trade name "Labco".

[3] When these studies were begun we still experienced considerable difficulties with bacterial synthesis of fecal riboflavin; hence, it was necessary to discard 1 or 2 weeks' metabolism data, and for this reason the periods of experimentation in the riboflavin studies were less than in the thiamine investigations. However, 2 to 3 weeks' balance studies yielded sufficient information to warrant the conclusions, presented in this paper.

stances, only tenths of a microgram. The change in body weights and food consumption during the experimental periods are presented in table 3. It will be noted that on the daily doses of 10, 20, and 50 µg. of thiamine and riboflavin there were only slight differences in gains of body weight; also, very small differences in increases of weight were apparent on the 100- and 1,000-µg. daily doses. The differences in efficiency of thiamine utilization, however, varied considerably with daily dose.

Thiamine utilization

Allowing for the thiamine excreted on a thiamine-deficient ration, the fecal excretions on the 5-, 10-, 20-, 50-, 100-, and 1-000-µg. daily doses represented 8.9, 5.0, 19.8, 28.9, 21.1, and 27.8% of the total daily intake, respectively. The urinary excretions of thiamine on the same increasing daily doses constituted 5.2, 3.7, 5.5, 5.6, 13.2, and 34.8%, respectively of the total intake. The thiamine absorbed is considered to be equal to the total intake minus the total excreted in the feces, corrected for the amounts excreted on a thiamine-deficient ration. The thiamine retained is regarded as equal to the thiamine absorbed less that excreted in the urine on a thiamine-deficient ration. The per cent thiamine utilized, then, equals

$$\frac{\text{thiamine retained}}{\text{thiamine absorbed}} \times 100$$

The riboflavin figures were calculated in similar fashion.

It is evident from table 1 that the fecal excretion of thiamine as per cent of the total intake rises with the increase of the dose, beginning with the 20-µg. daily dose; also, the rise in the urinary excretion of thiamine as per cent of the total intake does not begin until the 100-µg. dose. The 5, 10, 20, and 50-µg. daily doses were most efficiently utilized. Beginning with the 100-µg. dose, the efficiency of utilization begins to fall, and drops markedly on the 1,000-µg. dose to 51.9%. On the latter high dose of thiamine intake there is very poor retention, as evidenced by large proportions of urinary excretions of this vitamin. There is very little difference, however, in the ab-

TABLE 1

Influence of increasing doses of thiamine on efficiency of their utilization.

ANIMAL NUMBER ♂	METABOLISM PERIOD	DAILY THIAMINE INTAKE	TOTAL THIAMINE INTAKE	THIAMINE EXCRETED IN FECES [1]	THIAMINE ABSORBED		THIAMINE EXCRETED IN URINE [1]	THIAMINE RETAINED	THIAMINE UTILIZED	TOTAL EXCRETIONS OF THIAMINE IN FECES AND URINE	
	days	μg.	μg.	μg.	μg.	μg.	μg	μg.	%	μg.	%
1	26	5	130	10.2	119.8	92.1	7.3	112.5	93.9	17.5	13.4
2	26	5	130	12.8	117.2	90.1	6.2	111.0	94.9	19.0	14.6
3	13	10	130	6.1	123.9	95.3	5.2	118.7	95.8	11.3	8.7
4	13	10	130	6.9	123.1	94.7	3.5	119.6	97.2	10.4	8.0
5	13	20	260	47.9	212.1	81.6	13.6	198.5	93.6	61.5	23.5
6	13	20	260	55.0	205.0	78.8	15.0	190.0	92.7	70.0	26.9
7	13	50	650	190.8	459.2	70.6	37.8	421.4	91.6	228.6	35.2
8	13	50	650	185.3	464.7	71.5	34.4	430.3	92.6	219.7	33.8
9	26	100	2,600	549.3	2,050.7	78.9	338.1	1,712.6	83.5	887.4	34.1
10	26	100	2,600	574.8	2,025.2	77.9	347.9	1,677.3	82.3	922.7	35.5
11	26	1,000	26,000	7,087.6	18,912.4	72.7	8,544.4	10,368.0	54.8	15,632.0	60.1
12	26	1,000	26,000	7,353.6	18,646.4	71.5	9,508.4	9,138.0	49.0	16,862.0	64.9

[1] Corrected for the amount excreted on a thiamine-deficient ration.

sorption of thiamine on the 50-, 100-, and 1,000-µg. doses. The increased losses of thiamine in both feces and urine begin with the 20-µg. dose from 25% on this level of intake to 63% on the 1,000-µg. dose.

Riboflavin utilization

The fecal riboflavin excretions on the 5-, 10-, 20-, 50-, 100-, and 1,000-µg. daily doses, were 24.9, 27.8, 33.3, 22.6, 12.0, and 16.4% of the total intake, respectively. The urinary excretions of riboflavin on the same increasing daily doses were 16.6, 7.2, 4.7, 5.5, 11.0, and 9.8%, respectively. The above figures, and table 2 indicate that the low figures for absorbed riboflavin on the 5-, 10-, and 20-µg. daily doses are due to high fecal excretions of this vitamin, most probably due to bacterial synthesis. In this connection, it should be mentioned that no technique has as yet been perfected and, therefore, no precautions were taken to prevent bacterial synthesis while the feces were collecting in the metabolism cages between 5 P.M. and 8 A.M. the next day. That some bacterial synthesis in the feces occurred at night would appear from the fact that for the 5-, 10-, and 20-µg. daily doses of riboflavin, 19.3, 19.7, and 10.3% of the total intake of this vitamin were found in the fecal excretions on a riboflavin-deficient diet; while on the same daily doses of thiamine, 10.0, 9.1, and 3.0% of the total intake of this vitamin were excreted in the feces on a thiamine-deficient ration. With the increase of the daily dose of riboflavin, the amount excreted in the feces on a ration deficient in this vitamin became negligible. On 50-, 100-, and 1,000-µg. daily doses only 3.6, 0.6, and 0.05% of the total riboflavin intake, respectively, were excreted in the feces. The value of the balance studies on these higher doses then becomes relatively much more accurate and it is, therefore, interesting to note that, unlike the metabolism of thiamine, the efficiency of utilization on the large doses remains relatively constant and high. This is particularly true for the 1,000-µg. dose, the efficiency index being 88.3% for riboflavin and only 51.9% for thiamine.

TABLE 2

Influence of increasing doses of riboflavin on efficiency of their utilization.

ANIMAL NUMBER ♂	METABOLISM PERIOD	DAILY RIBOFLAVIN INTAKE	TOTAL RIBOFLAVIN INTAKE	RIBOFLAVIN EXCRETED IN FECES [1]	RIBOFLAVIN ABSORBED		RIBOFLAVIN EXCRETED IN URINE [1]	RIBOFLAVIN RETAINED	RIBOFLAVIN UTILIZED	TOTAL EXCRETIONS OF RIBOFLAVIN IN FECES AND URINE	
	days	μg	μg	μg	μg	%	μg	μg	%	μg	%
1	14	5	70	14.9	55.1	78.7	11.5	43.6	79.1	26.4	37.7
2	14	5	70	20.0	50.0	71.4	11.7	38.3	76.6	31.7	45.3
3	13	10	130	31.5	98.5	75.7	9.7	88.8	90.2	41.2	31.2
4	13	10	130	41.2	88.8	68.3	8.9	79.9	90.0	50.1	38.5
5	13	20	260	83.6	176.4	67.8	9.3	167.1	91.2	92.9	35.7
6	13	20	260	89.7	170.3	65.5	14.9	155.4	91.2	104.6	40.2
7	13	50	650	143.5	506.5	77.9	31.7	474.8	93.7	175.2	26.9
8	13	50	650	150.8	499.2	76.7	39.1	460.1	92.2	189.9	29.2
9	21	100	2,100	231.4	1,869.0	89.0	221.9	1,637.0	87.6	453.3	21.6
10	21	100	2,100	270.6	1,829.0	87.1	237.4	1,592.0	87.0	508.0	24.2
11	21	1,000	21,000	3,548.0	17,452.0	83.1	1,942.0	15,510.0	88.8	5,490.0	26.1
12	21	1,000	21,000	3,356.0	17,644.0	84.8	2,148.0	15,496.0	87.8	5,504.0	26.2

[1] Corrected for the amounts excreted on a riboflavin-deficient ration.

Since the excretions of thiamine and riboflavin in the feces and urine do not account for their total intake, the suggestions, of course, present themselves that excess of these vitamins are either stored in the tissues or destroyed during metabolism. To test the first hypothesis, two groups of male rats, six in each group, each about 35 days old and weighing approximately 100 gm., were given two different doses of thiamine and riboflavin for 30 days, supplementing the synthetic diet,

TABLE 3

Food consumption and changes in body weights on increasing doses of thiamine and riboflavin.

th.n.c. = thiamine negative control.
rf.n.c. = riboflavin negative control.

ANIMAL NUMBER (MALES)	DAILY DOSE OF THIAMINE	DAILY DOSE OF RIBO-FLAVIN	METAB-OLISM PERIOD	TOTAL FOOD CON-SUMPTION	CHANGE IN BODY WEIGHT
	$\mu g.$	$\mu g.$	days	gm	gm.
1	5	5	26	178.9	+ 14
1 — th.n.c.	0	5	26	125.3	— 1
2	5	5	26	193.9	+ 31
2 — rf.n.c.	5	0	26	156.8	— 12
3	10	10	13	147.1	+ 35
3 — th.n.c.	0	10	13	134.9	+ 39
4	10	10	13	148.3	+ 44
4 — rf.n.c.	10	0	13	113.9	+ 21
5	20	20	13	139.3	+ 43
5 — th.n.c.	0	20	13	104.2	+ 29
6	20	20	13	156.0	+ 39
6 — rf.n.c.	20	0	13	122.1	+ 27
7	50	50	13	132.7	+ 31
7 — th.n.c.	0	50	13	122.8	+ 20
8	50	50	13	128.4	+ 25
8 — rf.n.c.	50	0	13	94.6	+ 9
9	100	100	26	296.9	+ 97
9 — th.n.c.	0	100	26	130.9	— 18
10	100	100	26	305.7	+ 88
10 — rf.n.c.	100	0	26	182.1	+ 31
11	1,000	1,000	26	345.5	+ 115
11 — th.n.c.	0	1,000	26	111.6	— 13
12	1,000	1,000	26	312.8	+ 97
12 — rf.n.c.	1,000	0	26	173.0	+ 11

composition of which is given earlier in the paper; in addition, each animal received daily 20 µg. pyridoxine, 6 mg. choline chloride, and 200 µg. calcium pantothenate, One group received 100 µg. thiamine and 100 µg. riboflavin per animal per day and in the other group 1,000 µg. thiamine and 1,000 µg. riboflavin were given daily to each animal. At the end of the experiment the various tissues were dissected out, dried at 55° C. and analyzed for thiamine and riboflavin, according to techniques recently described (Sure and Ford, '41). It will be noted from table 4 that the concentration of these vitamins,

TABLE 4

Influence of 100 µg. and 1,000 µg. daily administration of thiamine and riboflavin for 30 days on distribution of these vitamins in various tissues.

	THIAMINE		RIBOFLAVIN	
TISSUE	100 µg. thiamine and 100 µg. riboflavin daily	1,000 µg. thiamine and 1,000 µg. riboflavin daily	100 µg. thiamine and 100 µg. riboflavin daily	1,000 µg. thiamine and 1,000 µg. riboflavin daily
	µg./gm.	µg./gm.	µg./gm.	µg./gm.
Liver	8.60	8.10	47.50	36.25
Kidney	9.70	8.40	62.50	66.25
Spleen	3.73	3.40	10.63	16.88
Heart	2.50	3.29	20.83	20.84
Lung	2.00	1.80	10.63	8.75
Brain	2.55	4.60	8.54	12.08
Testes	4.60	5.40	8.75	15.00
Stomach	2.75	1.35	6.25	7.92
Small intestines	1.80	1.80	12.50	6.57
Large intestines	2.87	1.37	7.50	7.50
Pancreas	1.76	1.20	8.84	10.00
Muscle	0.63	1.80	1.67	2.08

expressed as micrograms per gram on the dry basis, is not appreciably greater on the 1,000-µg. daily dose contrasted with the 1000-µg. daily allowance. In some tissues higher results are even indicated on the lower dose. In this respect, our findings are in accord with those of Leong ('37) who reported that maximum storage takes place on 90 µg. per day of vitamin B$_1$ intake. We found, however, considerably smaller excretions of thiamine in urine and much greater excretions of this vitamin in the feces on as high doses as 1,000 µg. daily;

also, we found that the fecal thiamine begins to rise on the 20-µg. rather than on the 150-µg. daily dose. In order to obtain information on the total thiamine and riboflavin stored in the body tissues, six additional male rats of similar age and weights as used in the other experiments outlined above were given 1,000 µg. thiamine and 1,000 µg. riboflavin daily for 30 days. They were then sacrificed and these vitamins were determined in their entire bodies. The results are given in table 5. It will be noted that after the intake of 30,000 µg. and an

TABLE 5

Thiamine and riboflavin content of animals which received 1,000 µg. of these vitamins daily for 30 days.

Animal number and sex	Dry weight of animal	THIAMINE		RIBOFLAVIN	
		µg./gm.	Thiamine content of animal	µg./gm.	Riboflavin content of animal
5290 — ♂	39.00	2.16	84.24	18.00	702.00
5290 — L♂	46.28	2.82	130.51	21.00	971.88
5291 — ♂	42.32	3.33	140.92	17.50	740.60
5292 — ♂	40.12	2.60	104.31	17.00	682.04
5292 — L♂	44.99	2.71	121.92	17.00	764.53
5302 — ♂	48.03	3.47	166.66	17.50	840.53
Average			124.76		782.64

equal amount of riboflavin, the average content per animal in its entire body was only 125 µg. thiamine and 783 µg. riboflavin. From such results it is quite apparent that the animal organism has only a very limited capacity for storing these two components of the vitamin B complex. How then can we account for the tremendous wastes in the metabolism of thiamine and riboflavin, particularly on the higher doses of intake? If oxidative or other destructive processes occur in the tissues, how could that be demonstrated experimentally? Although, in vitro we can never imitate vital phenomena occurring in vivo, attempts were made to determine if incubation at 37°C., or body temperature, of these vitamins with various body tissues for 24 to 48 hours, would show any destruction. Our results, presented in table 6, show appreciable destruction of

both thiamine and riboflavin by liver, lung, heart, stomach, small and large intestines. From the above results, it would appear then that at least an appreciable proportion of the losses of thiamine and riboflavin in metabolism is caused by their destruction in the body tissues after they have been utilized by such tissues for various functions.

TABLE 6

Influence of incubation at 37° C. on destruction of thiamine and riboflavin by body tissues.

Amount of thiamine incubated — 1000 μg.
Amount of riboflavin incubated — 1000 μg.

TISSUES	THIAMINE		RIBOFLAVIN	
	Incubation period		Incubation period	
	24 hours	48 hours	24 hours	48 hours
	Per cent destruction	Per cent destruction	Per cent destruction	Per cent destruction
Liver	0.0	14.0	5.0	20.0
Kidney	1.0	0.0	0.0	3.0
Spleen	7.0	...	7.5	25.0
Lung	8.5	28.0	25.0	25.0
Heart	10.5	17.0	28.0	25.0
Brain	1.0	...	0.0	10.0
Stomach	5.0	12.0	0.0	20.0
Small intestines	20.0	26.0	12.0	20.0
Pancreas	4.0	20.0	8.0	25.0
Thymus	0.0	...	0.0	...
Adrenals	17.0	...	0.0	...
Thyroids	0.0	...	12.5	...
Pituitary	6.0	...	2.5	...

SUMMARY

A quantitative metabolism study was made of the influence of increasing doses of thiamine and riboflavin on their efficiency of utilization. The daily doses used were: 5, 10, 20, 50, 100, and 1,000 μg. For thiamine, the 5-, 10-, 20-, and 50-μg. doses were most efficiently utilized. On higher levels of intake the efficiency of utilization decreases, there being a marked drop on the 1,000-μg. daily dose. The efficiency of utilization on the 50-, 100-, and 1,000-μg. daily doses were 92.1, 82.9, and 51.9%, respectively.

It is impossible at present to evaluate the efficiency of utilization of the 5-, 10-, and 20-µg. doses of riboflavin, because the fecal excretions indicate some bacterial synthesis. However, on the higher intakes of 50-, 100-, and 1,000-µg. doses, the total fecal riboflavin excretions are insignificant in terms of the total intake of this vitamin, hence the figures of 76 to 88% utilization on these higher doses are significant, and show that massive doses of riboflavin are much more economically utilized than equal intakes of thiamine. The total thiamine and riboflavin excretions do not account for the total intake of these vitamins. Neither could they be accounted for by storage in the animal tissues. However, incubation at 37° C. for 22 to 48 hours showed appreciable destruction of thiamine and riboflavin by liver, lung, heart, stomach, small and large intestines. It would appear then that part of the losses of thiamine and riboflavin in metabolism are caused by their destruction in the tissues.

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STUDIES ON CAROTENOID METABOLISM

IV. THE EFFECT OF VITAMIN A INTAKE ON THE CAROTENOID AND
VITAMIN A CONTENT OF THE EGGS, LIVER, BLOOD
AND BODY FAT OF HENS

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TWO FIGURES

Not only has it been shown that the transfer of vitamin A to the milk is greatly augmented when massive doses of this vitamin are administered to cows, but also it has been demonstrated that this is accompanied by a concomitant decrease in the butter carotene in spite of the fact that the intake of the carotenoids remains essentially constant (Deuel, Halliday, Hallman, Johnston and Miller, '41; Deuel, Hallman, Johnston and Mattson, '42). In a later study, Mattson and Deuel ('43, a) found that a similar depression in blood and liver carotenoids accompanied the administration of large doses of vitamin A in baby chicks although the pigments here are chiefly the carotenols such as lutein (xanthopyll). In the present study, the investigations have been extended to adult chickens to determine whether an increased vitamin A intake would result in an augmented excretion of vitamin A in the eggs along with a decreased pigment content. Studies have also been made of the egg production and hatchability of the eggs produced on the several diets which will be reported elsewhere.

In eggs vitamin A is present almost entirely in the yolk. The quantity has been shown to vary with diet but the average level is given by Sherman ('41) as 2,500 to 5,000 I. U. per 100 gm. of yolk which would give an average value of 600 I. U. (400 — 800 I. U.) per egg where the yolk weighs approximately 16 gm. Russell and Taylor ('35) report values of 500 to 800 I. U. per 16 gm. yolk in eggs obtained from chickens on several diets. Other results based on bioassays are somewhat lower. Ellis et al. ('32) report values of about 500 Sherman-Munsell units per egg (31 S. M. units per gram yolk) in a diet fortified with cod liver oil while Koenig et al. ('35) give values averaging 33 and 20 S. M. units per gram of yolk in low and high egg producers respectively. Pale yolked eggs on carotene-free diets containing cod l'ver oil contained 25 S. M. units per gram of yolk. Bearse and Miller ('37) obtained values of 10 to 28 I. U. per gram of yolk, the higher levels being found with an increased intake of vitamin A. Sjollema and Donath ('40) noted a maximum of 321 I. U. per egg in chickens fed on a yellow corn-alfalfa diet although this was lowered approximately 50% when the alfalfa was omitted. Cruickshank and Moore ('37) have found that the A content of eggs was doubled by the incorporation of 10% of cod liver oil while it was increased five times by the administration of an A concentrate. The augmentatory effect of diet is also reported by Sherwood and Fraps ('34) and Bethke, Kennard and Sassaman ('27). Hauge and Zscheile ('42) have shown that the vitamin A content of eggs dried by the Mojonnier spray drier is destroyed less than 5%; they report a value of 44 I. U. per gram of dried whole egg which would correspond with approximately 33 I. U. per gram of yolk.

The effect of the carotenoid intake on the color of egg yolk has been recognized since the classical work of Palmer and Kempster ('19). More recently it has been shown that the amount of carotenols deposited varies with the source of the xanthopylls as well as with the amount ingested. Also the content of carotene and cryptoxanthine was found to vary with different hens and it could not be increased with in-

creased intake (Hughes and Payne, '37). In fact only 2.5% of the carotene in alfalfa was shown to be deposited in the yolk. Sjollema and Donath ('40) found that the carotene made up only approximately 7% of the total carotenoids in the yolks of an alfalfa or yellow corn-alfalfa diet while it comprised an average of 11% on a diet in which the pigments were derived only from yellow corn.

On the other hand the depressing effect of a very high intake of vitamin A on the pigmentation of egg yolks has not been recognized. It is demonstrated here that this factor may have as profound an effect as that of the exogenous source of these pigments.

EXPERIMENTAL

The experiments were carried out on six groups of white Leghorn chickens each of which was made up of twenty-seven hens and three roosters. With two groups of chickens, the vitamin A supplements were increased after 3 months so that a control group and seven levels of vitamin A feeding are reported here. They were maintained throughout on a basal diet high in carotenoids which had the following composition per 100 lbs:

Ground yellow corn	34.90 lbs.
Recleaned ground oats	5.00 lbs.
Ground wheat	24.90 lbs.
Flaky white bran	17.95 lbs.
Fish meal	5.48 lbs.
Dehydrated alfalfa	5.00 lbs.
Soybean meal	2.50 lbs.
Dried whey	2.00 lbs.
Oyster shell flour	1.00 lbs.
Fine oyster shell grit	0.745 lbs.
Sodium chloride	0.50 lbs.
Manganese sulfate	0.025 lbs.

To this was added 22,500 units of vitamin D_3
(50 units per 100 gm. of feed).

The basal diet was fed to all the groups during the basal period and to the control group throughout the experiment. It contained approximately 10 mg. of carotenoids per pound

of food. Sufficient high potency shark liver oil (approximately 20,000 I. U. per gram) was mixed with the basal diet to give the food an additional potency of 1,000, 2,000, 15,000, 30,000, 60,000, 100,000, and 200,000 I. U. per pound. To avoid loss of vitamin A by prolonged storage, the diets were made up at frequent intervals by mixing the basal diet and the required amount of vitamin A oil. The food consumption of the chickens approximated ¼ lb. per day; therefore the average daily intake of vitamin A per chicken was approximately one-fourth of the above values. The basal period began on August 21, 1942 and continued until November 2nd except with group 7 where it was terminated on October 15th. At the termination of the basal period, the supplements were mixed with the diets and fed until the termination of the experiment on April 24, 1943. After 75 days on the supplement, groups 3 and 4 were discontinued, and two new groups were formed (6 and 8) by placing approximately 50% of the chickens in each of the original groups in each of the new groups. These were continued on the new levels of vitamin A (60,000 and 200,000 I. U.) until the termination of the test.

The tests were carried out at the poultry ranch associated with the Emeryville Plant of the California Packing Corporation and were supervised by one of us (A. R.). Twelve eggs from each group from the previous days' production were forwarded to Los Angeles by express each Saturday and analyses were usually started on the following Tuesday.

The analyses for vitamin A and carotenoid pigments were made on aliquots from the pooled samples of the yolks from each group. After determining the total weights of the whole egg, the yolks·were separated mechanically, weighed, and homogenized in a Waring blendor. Weighed samples were saponified in 50% alcohol with 5 ml. of 40% KOH for 30 minutes and then transferred to an all glass extraction apparatus where they were extracted for 3 hours with low boiling Skelly Solve. Saponification and extraction were carried out in a dark room where the light globes were covered with a special lacquer [1]

[1] This lacquer was kindly furnished us by Mr. A. Cherkin of the laboratories of Don Baxter, Inc., Glendale, California.

inasmuch as it has been shown that marked destruction of vitamin A takes place not only in direct sunlight but also in diffuse daylight or even in light from mazda lamps (Mattson and Deuel, '43, b). The petroleum ether extract was evaporated to small volume, filtered into 50 ml. volumetric flasks, absolute isopropanol was added and the final volume of solvents adjusted so that the concentration was 75% isopropanol and 25% petroleum ether. The absorption was determ'ned on the Beckman spectrophotometer at 326 mµ (for vitamin A) and 446 mµ (for carotenoids). The value for absorption of vitamin A was corrected for that of the carotenoids by multiplying $\log \frac{I_0}{I_{446}}$ by the factor 0.06. A conversion factor of 2060[2] was used to determine I. U. of vitamin A per gram of yolk from the extinction coefficients.[3]

Since xanthopyll makes up the largest portion of the carotenoids, the concentration of total carotenoids was calculated from the absorption coefficient at 446 mµ divided by 0.252[4] which was determined on a pure lutein sample[5]. In cases where vitamin A was determined on single eggs, a homogenous suspension was made with 95% alcohol in the Waring blendor and appropriate aliquot samples were used for saponification.

At the conclusion of the experiments, six hens of each group were killed by bleeding following a 72-hour fast. Determinations of carotenoids and vitamin A were made on the blood plasma, liver and body fat. The liver was homogenized with 95% ethyl alcohol in the Waring blendor and stored several days in the refrigerator until determinations could be made. No decrease was noted in samples kept for a week under such conditions.

[2] The spectrophotometer was checked at frequent intervals with potassium chromate (Morton, '42). The factor, 2060, was obtained by calibration with reference cod liver oil.

[3] Vitamin A $_{(gm.\ yolk)} = \dfrac{(\log \frac{I_0}{I_{326}} - 0.06 \log \frac{I_0}{I_{446}}) \times \text{dilution}}{\text{Wt. of aliquot}} \times 2060$

[4] Carotenols per gram yolk $= \dfrac{\log \frac{I_0}{I_{446}} \times \text{dilution}}{\text{Wt. of aliquot} \times 0.252}$

[5] This sample of pure lutein was kindly furnished us by Dr. L. Zechmeister of the California Institute of Technology.

RESULTS

The average values for vitamin A and carotenoids in the yolks obtained over the 24-week period are given in table 1 while the fluctuations are evident from figures 1 and 2.

There was no alteration in pigment content of the yolks where 1,000 or 2,000 I. U. of vitamin A were incorporated per pound of food. A definite decrease was found in group 4 (15,000 I. U. per pound) and a progressively greater decrease with succeeding groups to 25% of the original level with the highest intake of vitamin A (group 8).

On the other hand, no increase in the vitamin A content of eggs obtained in group 4 where a suppressing effect on the carotenoid pigments had been noted. A small increase was found in group 5 and a gradual rise in the grand average to 121 I. U. per gram of yolk for group 8. During the course of the experiments, averages during individual weeks as high as 158 I. U. were found; in the last week of the test, where determinations were made separately on eggs from ten different chickens in group 8, an average of 141.7 I. U. was noted, the maximum being 226.3 and the minimum 91.8 I. U. per gram of yolk. The total carotenoids in separate eggs averaged 8.17 micrograms per gram of yolk, the variations being from 14.1 to 3.55 micrograms.

The results of the analyses of the liver, plasma, and body fat of six chickens from each group (except groups 3 and 4) are recorded in table 2.

The carotenoids in the serum and liver are apparently progressively lowered in groups 5 to 8 over the control group and the group receiving 1,000 I. U. per gram. Because of the small number of observations, these results are statistically significant only with the two groups receiving the highest level of supplement. On the other hand, the carotenoids show a marked lowering in the body fat only in group 7; in group 8, where the suppression of carotenoids in the liver and serum had been the greatest, no decrease was noted.

The vitamin A content of the serum is constant although there is some indication that it may be somewhat elevated

TABLE 1

The carotenoid and vitamin A contents of eggs during a basal period (Period I) when only the basal diet was fed and during an experimental period (Period II) when different amounts of vitamin A were incorporated in the diet.

GROUP NO.	VITAMIN SUPPLEMENT PER POUND FOOD	NO. OF SAMPLES		CAROTENOIDS IN YOLK			VITAMIN A IN YOLK			VITAMIN A PER EGG (18 GM. YOLK)	
		Period I	Period II	Period I	Period II Amount¹	M.D.: S.E.M.D.³	Period I	Period II Amount¹	M.D.: S.E.M.D.³	Period I	Period II
	I.U.			*γ/gm.*	*γ/gm.*		*I.U./gm.*	*I U/g.m.*		*I.U.*	*I.U.*
1	0	3	23	38.3	32.3 ± 2.0		36.6	36.8 ± 0.7		840	844
2	1,000	3	23	36.1	29.4 ± 1.5	1.16	41.6	46.7 ± 0.8		803	842
3	2,000	3	9	40.2	33.0 ± 1.7	0.27	46.8	46.3 ± 1.3		844	835
4	15,000	3	11	36.8	25.3 ± 0.7	3.30	47.9	48.4 ± 0.7	1.62	862	872
5	30,000	2	23	39.0	21.7 ± 1.1	4.65	48.6	54.6 ± 0.8	7.35	875	982
6	60,000³		12	(29.2)	12.9 ± 0.8	9.12	(47.4)	62.3 ± 3.3	5.56	(854)	1122
7	100,000	3	24	38.0	9.7 ± 0.6	10.82	44.3	99.3 ± 3.3	15.53	798	1795
8	200,000³		13	(29.2)	8.4 ± 0.5	11.62	(47.4)	120.7 ± 7.1	10.38	(851)	2170

¹ Including the standard error of the mean calculated as follows: $\sqrt{\Sigma d^2/n}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations. These averages for carotene in all groups on Period II and for vitamin A in groups 6, 7 and 8 exclude the values for the first 3 weeks of supplement.

² Mean Difference: Standard Error of Mean Difference calculated as follows: $M.D./\sqrt{(S.E.M._1)^2 + (S.E.M._2)^2}$. When this exceeds 3.00 the results are considered significant. The comparisons are made in all cases with the control group.

³ The values in parentheses are the average values of groups 3 and 4 for the 8-week period immediately preceding. After that groups 6 and 8 were started with the hens comprising those of groups 3 and 4.
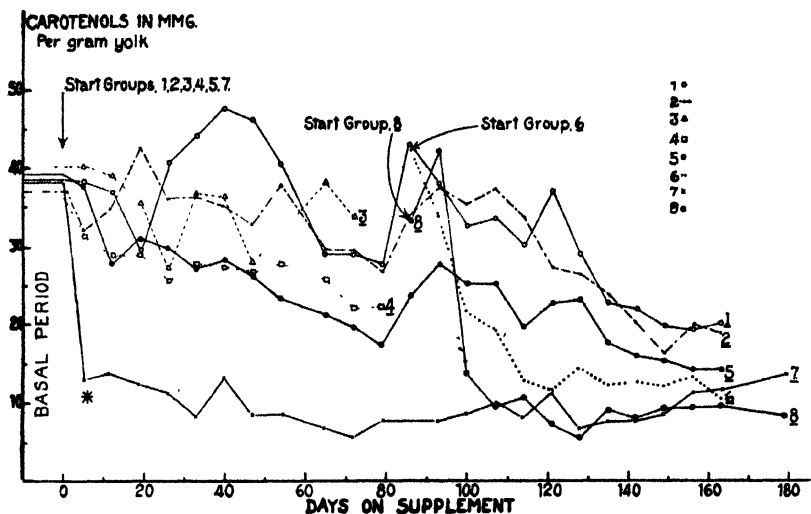
Fig. 1 The carotenol content in micrograms per gram of yolk. The first measurement (starred) in the curve for group 7 only was made at an interval of 23 days after the start of the supplement.
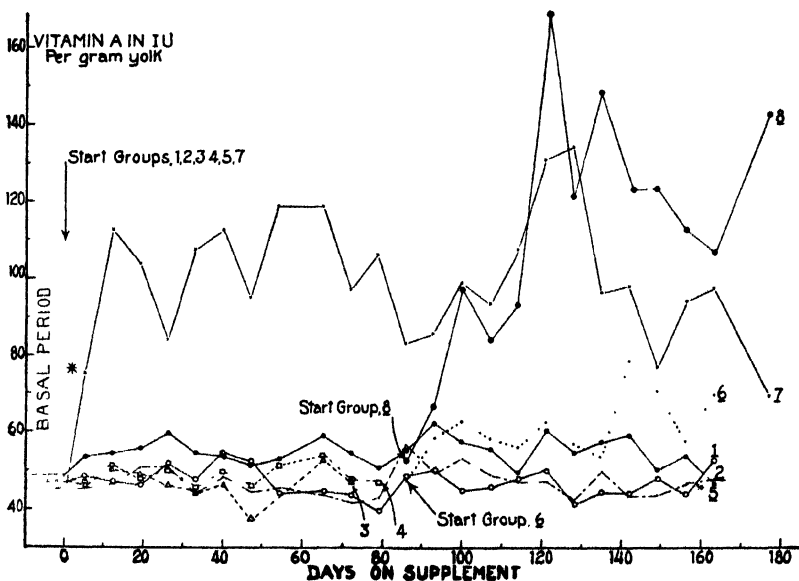
Fig. 2 The vitamin A in I.U. per gram of yolk. The first measurement (starred) in the curve for group 7 only was made at an interval of 23 days after the start of the supplement.

in group 8. On the other hand, there seems to be no further increase in storage of vitamin A in the liver when the supplement is greater than 30,000 I. U. of vitamin A although the maximum values become progressively higher. The maximum storage was noted in one of the hens of group 7 where 411,000 I. U. were stored in the whole liver.

The body fat shows a marked rise in vitamin A storage which, however, is only slight in the 30,000 I. U. group but reaches an average about eight times the basal level in the 200,000 I. U. group. In one chicken here a maximum vitamin A content of 340 I. U. was found per gram of fat compared with an average value for the control group of 32.3 I. U. per gram.

The hens were in good condition throughout the test although those receiving the high levels of vitamin A had a different appearance because of the loss of carotenols from the

TABLE 2

The carotenoid and vitamin A content of the serum, liver and body fat of hens which had received the basal diet only for 8 months (group 1) or with added vitamin A supplements for approximately 3 months (groups 6 and 8) or for approximately 6 months (groups 2, 5 and 7).

GROUP NO [1]	BODY WEIGHT	CAROTENOIDS			VITAMIN A		
		Serum	Liver	Fat	Serum	Liver	Fat
	gms	$\gamma/100cc.$	$\gamma/gm.$	$\gamma/gm.$	*I. U./gm.*	*I. U./gm*	*I. U./gm*
1	1551	143.2[2]	7.8	9.5	463	370	32.3
		(212.8–60.3)	(14.3–6.0)	(15.8–4.5)	(350–372)	(943–78)	(52.4– 19.4)
2	1603	218.0	11.7	4.5	554[2]	665	23.4
		(501.5–31.8)	(21.3–5.4)	(6.7–0.0)	(801–346)	(1013–70)	(37.7– 15.5)
5	1635	111.4	6.7	5.9	445	4592	42.2
		(183.0–51.8)	(11.7–4.4)	(7.8–3.0)	(517–388)	(9043–406)	(58.3– 32.6)
6	1656	105.8	6.3	6.1	580	6940	59.3
		(163.5–29.6)	(10.6–2.2)	(8.1–4.5)	(1320–350)	(10070–2780)	(77.0– 41.6)
7	1585	62.6	4.0	2.3	443	4654	92.7
		(205.2–11.8)	(8.2–1.5)	(5.1–0.3)	(650–262)	(12825–1100)	(166.0– 25.0)
8	1602	37.0	2.7	8.0	689	4626	222.6
		(68.6–11.6)	(4.5–0.8)	(16.1–2.8)	(1076–464)	(13888–1220)	(340.0–167.6)

[1] Six chickens in all groups except group 5 where there were five. The figures in parentheses represent the maximum and minimum values in each group.

[2] Five determinations only.

combs and wattles. There was no evidence of an adverse effect on egg production; in fact, there is some indication of an increased production although the experiments are not sufficiently extensive to make this certain. No gross differences in food consumption were noted.

DISCUSSION

Large doses of vitamin A depress the carotenol content of hen's egg in the same way that the carotene content of cow's milk is lowered (Deuel et al. '42). In the experiments on cows, a daily intake of 230,000 or 470,000 I. U. of vitamin A brought a lowering in carotene although no rise in vitamin A content of the milk obtained. In the case of hens a similar phenomenon was noted; the carotenoid content of the eggs of group 4 which received 15,000 I. U. of vitamin A per pound of food was significantly lowered although the vitamin A in the eggs was not increased.

The carotenol content of the serum is also progressively lower with increasing doses of vitamin A in the diet. No marked effect was noted in the liver, however, except with groups 7 and 8 although the results on groups 5 and 6 might be significantly lower if more experiments were available. Also, the most marked lowering in carotenoid content of body fat obtained in group 7 where the values are about 25% of the levels found in the control group. The mean figure for group 8, however, shows no lowering from the control level although this group had exhibited the greatest lowering in the pigment content of the serum and liver. The discrepancy between groups 7 and 8 may be explained because of the difference in the length of time during which the supplement was administered. The analyses on group 7 were obtained from tissues of chickens which had received the vitamin A supplement for $6\frac{1}{2}$ months while the results on group 8 were obtained after the birds had received the food containing 200,000 I. U. of vitamin A per pound for only 3 months. Prior to that they had been receiving supplements of 2,000 or 15,000 I. U. per pound. These data would seem to illustrate the slow

turnover of the carotenoids in body fat as contrasted with the much more rapid response of the liver, serum and eggs.

The vitamin A content of the eggs was slightly increased in the 30,000 I. U. group and progressively higher as the supplements were increased. The maximum average value of 158 I. U. per gram of yolk found in group 8, corresponds with 2,840 I. U. of vitamin A per egg. The highest value of 226 I. U. found in a single egg in group 8 accounts for 3,850 I. U. of vitamin A. Still a higher concentration was obtained in an ovum from one of the hens which was sacrificed where a value of 250 I. U. of vitamin A per gram was noted.

The maximum lowering in carotenols requires 20 to 30 days and continues to be maintained for at least 6 months. About the same interval is required for the vitamin A to reach the higher level in the yolk. There is a marked fluctuation in the mean value obtained on different weeks after the higher level has been reached. This may be due to an alteration in the vitamin A deposited or to the variation in the sources of the eggs at different times. Since it is evident from our examination of the eggs of different hens that a marked variation may be obtained, a difference in the mean value may very well be caused by the inclusion or exclusion of some of the eggs containing the maximum levels of vitamin A. There is no evidence that a tolerance for ingested vitamin A may develop in 6 months with a resultant drop in the vitamin A excreted in the egg.

SUMMARY

The administration of large amounts of vitamin A to hens receiving a basal diet high in carotenoids resulted in a marked and progressively greater suppression in the pigment content of the egg yolk as the vitamin A intake was increased. On the diet in which 15,000 I. U. of vitamin A was incorporated per pound, the lowering was significant, while it was decreased to only 25% of the basal level in chickens receiving the basal diet containing 200,000 I. U. of vitamin A per pound of food. A similar effect was noted in the blood and liver; it was also strikingly evident in the body fat of group 7 which

had received a supplement of 100,000 I. U. of vitamin A for over 6 months but not in the case of group 8 which received twice the level of vitamin A for 3 months. This is considered to be the resultant of the sluggish turnover of the carotenoids in body fat.

The vitamin A in the yolk was unchanged from the average control level of 46.8 I. U. per gram when 1,000, 2,000 or 15,000 I. U. of this vitamin were added per pound of food. With higher doses of vitamin A in the diet, significantly increased levels were found in the yolks, the values respectively being the following: 30,000 I. U. group, 54.6; 60,000 I. U. group, 62.3; 100,000 I. U. group, 99.3 I. U.; and with the 200,000 I. U. group, 120.7 I U. The maximum level found was 226 I. U. There was no evidence of the development of a tolerance for vitamin A with a resultant decrease in the vitamin A excreted during a 6-month period.

The vitamin A is progressively increased in the body fat with the higher intake in the diet. This was augmented from a value of 32.3 I. U. per gram in the control group to a mean value of 222.6 I. U. per gram in the group receiving 200,000 I. U. per pound of food. Maximum levels of vitamin A in the liver were found in the 60,000 I. U. group and no greater increases were noted when the intake was further augmented. The vitamin A in the serum was unaltered except that a slight increase probably occurred when the supplement was fed at the highest level.

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The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award will be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by February 1, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

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HENRY A. MATTILL,
State University of Iowa,
Iowa City, Iowa

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Chairman, Nominating Committee

MEAD JOHNSON AND COMPANY
'B-COMPLEX' AWARD

Nominations are solicited for the 1944 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B Complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1943 must be in the hands of the Secretary by January 10, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
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SECRETARY, AMERICAN INSTITUTE OF NUTRITION

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